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# VOJNOSANITETSKI PREGLED

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Photographies of the Chernobyl nuclear power plant (top) and Fukushima Daiichi nuclear power plant (bottom) after accidents in 1986 and 2011, respectively.

This spring marks 30 and 5 years, respectively, of these disasters. In this issue of the Journal Djurović et al. discuss what we have learned and what we have done in the meantime (see p. 484–90).

Fotografije nuklearnih elektrana u Černobilju (gore) i Fukušimi (dole) posle akcidenata koji su se dogodili 1986, odnosno 2011. godine.

Ovog proleća obeležava se 30, odnosno 5 godina od pomenutih katastrofa. U ovom broju časopisa, Djurović i sar. komentarišu pouke proistekle iz tih događaja, i daju kritički osvrt na aktivnosti preduzete u međuvremenu (vidi str. 484–90).



## Intraoperative hemorrhage as a complication of cesarean myomectomy: analysis of risk factors

Intraoperativno krvarenje kao komplikacija miomektomije tokom carskog reza: analiza faktora rizika

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### Abstract

**Background/Aim.** Cesarean myomectomy is a controversial issue. It was considered relatively contraindicated for many years due to increased risk of intraoperative hemorrhage. Recent studies showed that cesarean myomectomy in some women may not be associated with increased morbidity. The aim of the study was to determine the causes and risk factors for intraoperative hemorrhage in patients subjected to cesarean myomectomy. **Methods.** This retrospective study included women subjected to cesarean myomectomy, divided into the study group of 36 patients in whom intraoperative hemorrhage was registered, and the control group of 66 patients in whom it was absent. The following parameters were analyzed: age, parity, gestational age of delivery, indications, type and duration of cesarean section, surgeon's experience, type, localization, size and number of myomas, number of incisions on uterus and neonatal birth weight. **Results.** There was a significant difference between the groups in terms of the type and size of myomas ( $p = 0.007$  and  $p = 0.000$ , respectively) and duration of the surgery ( $p = 0.000$ ). The size of the defect resulting from myoma enucleation and speed of suturing it have significant influence on the occurrence of intraoperative hemorrhage. In our study, operation on the patients of the study group lasted 14.53 minutes longer and their myomas were 39 mm bigger compared to the controls, with no difference in surgical experience of the obstetricians ( $p = 0.111$ ). **Conclusion.** Cesarean myomectomy is associated with an increased risk of hemorrhage. Therefore, it would be advisable to discuss the hemorrhage and transfusion risks with patients with large multiple and intramural myomas before making decision to perform cesarean myomectomy. Those who perform cesarean myomectomy should be prepared to manage intraoperative hemorrhage during surgery in cases they encounter it.

### Key words:

cesarean section; uterine myomectomy; risk factors; uterine neoplasms; myoma; leiomyoma; hemorrhage; intraoperative complications.

### Apstrakt

**Uvod/Cilj.** Miomektomija tokom carskog reza je predmet rasprava. Ona se dugo godina smatrala relativno kontraindikovanom zbog povećanog rizika od intraoperativnog krvarenja. Nedavne studije su, međutim, pokazale da miomektomija tokom carskog reza kod nekih žena ne mora biti povezana s povećanim morbiditetom. Cilj istraživanja je bio da se utvrde uzroci i faktori rizika od nastanka intraoperativnog krvarenja kod žena podvrgnutih miomektomiji tokom carskog reza. **Metode.** Ova retrospektivna studija obuhvatila je žene podvrgnute miomektomiji tokom carskog reza, koje su bile svrstane u dve grupe: studijsku, koju je činilo 36 ispitanica kod kojih je ustanovljeno intraoperativno krvarenje i kontrolnu, koju je činilo 66 žena kod kojih krvarenje nije zabeleženo. Analizirani su sledeći parametri: starost i paritet, nedelja gestacije porođaja, indikacija, vrsta i trajanje carskog reza, iskustvo hirurga, tip, lokalizacija, veličina i broj mioma, broj rezova na materici i težina deteta na rođenju. **Rezultati.** Utvrđena je statistički značajna razlika između grupa u pogledu tipa i veličine mioma ( $p = 0,007$  odnosno  $p = 0,000$ ) i trajanja operacije ( $p = 0,000$ ). Veličina defekta nastalog nakon enukleacije mioma i brzina njegovog ušivanja statistički su značajno uticali na nastanak intraoperativnog krvarenja. Operacija žena studijske grupe trajala je 14,53 minuta duže nego operacija ispitanica kontrolne grupe, a njihovi miomi su bili 39 mm veći od mioma ispitanica kontrolne grupe. Nije bilo statistički značajne razlike u hirurškom iskustvu akušera koji su operisali žene obe grupe ( $p = 0.111$ ). **Zaključak.** Miomektomija tokom carskog reza povezana je s povećanim rizikom od intraoperativnog krvarenja. Stoga, bilo bi preporučljivo obavestiti žene s velikim multiplim i intramuralnim miomima o rizicima krvarenja i transfuzije pre donošenja odluke o miomektomiji tokom carskog reza. Oni koji vrše miomektomiju tokom carskog reza trebalo bi da budu spremni da zbrinu intraoperativno krvarenje ukoliko do njega dođe tokom operacije.

### Ključne reči:

carski rez; materica, miomektomija; faktori rizika; materica, neoplazme; miom; leiomiom; krvarenje; intraoperativne komplikacije.

## Introduction

A high percentage of cesarean sections (CS) in women with fibroids was noticed at the first half of the 20th century<sup>1</sup>. Cesarean myomectomy (CM) is still a controversial issue<sup>2</sup>. It was considered relatively contraindicated for many years due to increased risk of intraoperative hemorrhage which may even require postpartum hysterectomy<sup>3,4</sup>. Recent studies showed that CM in some women may not be associated with increased morbidity<sup>5,6</sup>. The reasons for reviewing the attitudes of CM are relatively rare, but very serious myoma complications during puerperium, which might require surgical treatment and even postpartum hysterectomy<sup>1</sup>. CM enables performing two operations in one laparotomy, as well as preservation of the uterus, while avoiding risks of relaparotomy and repeated anesthesia, complications of myomas during puerperium and subsequent pregnancies<sup>7,8</sup>.

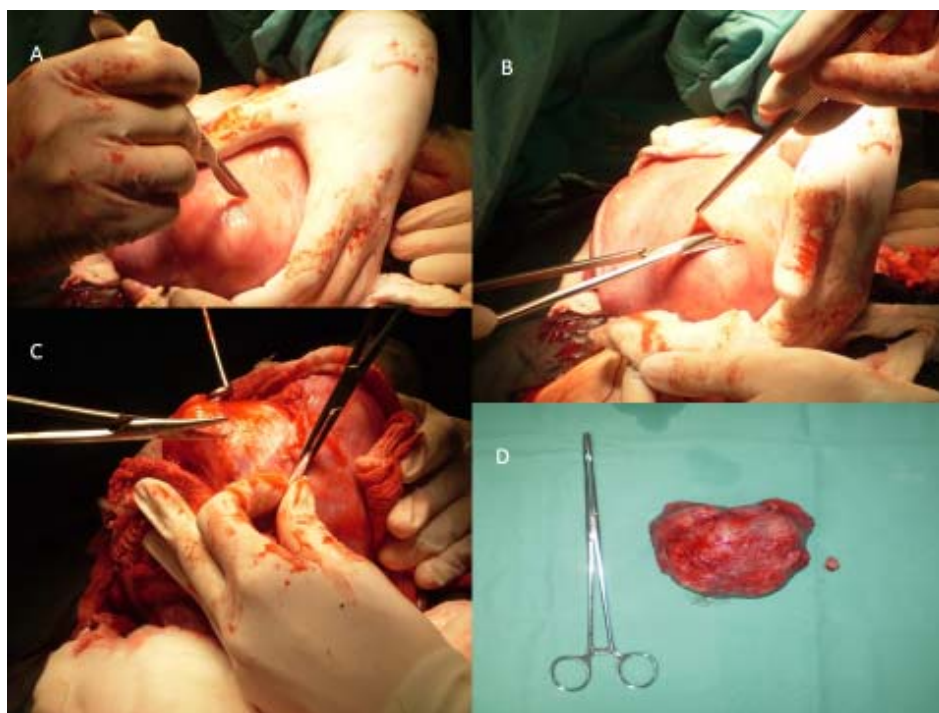
The aim of the present study was to determine the incidence and causes of intraoperative hemorrhage in patients subjected to CM, as well as to define risk factors for intraoperative hemorrhage in these patients.

## Methods

This retrospective case control study included women who had undergone CM during a 5-year period in a single teaching hospital. Criteria for exclusion from the study were: placenta *previa* or placental abruption, congenital or acquired coagulopathy, multiple pregnancy, and additional surgery during CM (other than myomectomy). The study was approved by institutional Ethics Committee. All the 102 patients

included gave the informed consent for the operation. CM indications included: patients' wish, symptomatic or degenerative myoma and tumor *previa*. Myomectomy was always performed by sharp dissection of myoma (Figure 1). No tourniquet and electrosurgery were used. The study group consisted of the patients in whom intraoperative hemorrhage was registered, 36 of them (group I). The controls (group II) included 66 patients with no intraoperative hemorrhage. The following parameters were analyzed: age, parity, gestational age at delivery, indications for CS, type of CS (emergency or elective), duration of CS, surgeon experience, type, localization, size and number of myomas, number of incisions on uterus and neonatal birth weight. The indications for CS were defined based on the primary indication for surgery. The duration of surgery was calculated in minutes from skin incision to skin closure. Type, localization and the number of myomas were assessed from the operative reports. Myoma size was determined by the largest diameter of the myoma measured by the pathologist. In cases of multiple myomas, the diameter of the largest fibroid was taken into account. Intraoperative hemorrhage was defined by the results from the surgical operative note, the need to administer carboprost during surgery, intraoperative transfusion of heterologous or autologous blood, and based on the reduction of hemoglobin levels greater than 40 g/L and/or reduction of hematocrit values greater than 10%.

Statistical comparisons were made between the control and study groups of patients. Data were analyzed using the statistical software SPSS version 20.0. Parametric data, after controlling normal distribution were compared by using the Student's *t*-test. For comparisons of a difference in terms of



**Fig. 1 – Myoma enucleation during cesarean myomectomy: A) Linear incision over the uterine serosa by scalpel; B) Myoma dissection using sharp Metzenbaum scissors; C) Myoma hooked and extracted from its capsule; D) Enucleated myoma.**

myoma size and localization among groups we used Likelihood Ratio. In order to evaluate the predictive accuracy of myoma size in the occurrence of intraoperative hemorrhage we used receiving operating characteristics (ROC). The unique level of significance throughout the study was 0.05.

## Results

The incidence of intraoperative hemorrhage in our study was 35.29%.

The groups did not differ by age, parity, gestational week of delivery and neonatal body weight, as shown in Table 1. None of the newborns had birth weight greater than 4,000 g. The largest number of patients in both groups were primiparas in term gestations.

Features of myomas are shown in Table 2. There was a highly significant difference between the groups in terms of the type and size of myomas, without difference in myoma number and localization. The most common myoma type was multiple in the study group (47.2%), and subserous (51.5%) in the control group. Anterior myomas were the most common in both groups, accounting for 50.0% of myomas in the study and 65.2% in the control group, respectively. The study group patients had on average 39.11

mm bigger myomas. Impact of myoma size on the occurrence of intraoperative hemorrhage was further analyzed by ROC. The area under ROC (AUROC) curve for the intraoperative hemorrhage was 0.825 (Figure 2, Table 3).

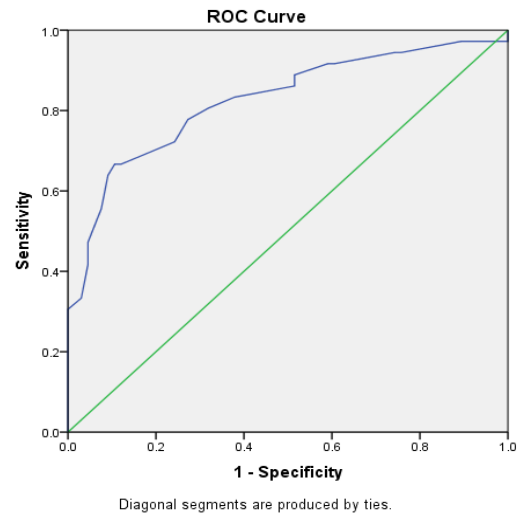


Fig. 2 – Receiving operating characteristic (ROC) curve for the sensitivity of myoma size in the occurrence of intraoperative hemorrhage.

Table 1

Patients' characteristics			
Characteristics	Study group ( $\bar{x} \pm SD$ )	Control group ( $\bar{x} \pm SD$ )	<i>p</i>
Age (years)	34.19 ± 5.24	34.68 ± 4.17	0.608
Parity (n)	1.17 ± 0.45	1.17 ± 0.41	1.000
Gestational age (weeks of gestation)	39.08 ± 1.95	38.94 ± 1.85	0.713
Neonatal birth weight (gr)	3179.17 ± 535.77	3231.82 ± 566.61	0.649

Study group – patients with intraoperative hemorrhage;

Control group – patients without intraoperative hemorrhage.

Table 2

Myoma characteristics			
Characteristics	Study group	Control group	<i>p</i>
Number of myoma, $\bar{x} \pm SD$	1.69 ± 0.92	1.88 ± 1.47	0.497
Myoma size (mm), $\bar{x} \pm SD$	80.92 ± 40.85	41.80 ± 18.24	0.000**
Myoma type, n (%)			
pedunculated	5 (13.9)	4 (6.1)	
subserous	7 (19.4)	34 (51.5)	0.006**
intramural	7 (19.4)	4 (6.1)	
multiple	17 (47.2)	24 (36.4)	
Myoma localization, n (%)			
fundal	4 (11.1)	4 (6.1)	
anterior wall	18 (50.0)	43 (65.2)	0.384
posterior wall	8 (22.2)	15 (22.7)	
isthmicocervical	3 (8.3)	2 (3.0)	
cornual	3 (8.3)	2 (3.0)	
Total, n (%)	36 (100.0)	66 (100.0)	

Study group – patients with intraoperative hemorrhage;

Control group – patients without intraoperative hemorrhage.

\* *p* < 0.05; \*\* *p* < 0.01.

Table 3

Area under receiveig operating characteristic (AUROC) curve: test results for myoma size				
AUROC	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval	
			lower bound	upper bound
0.825	0.046	0.000	0.734	0.916



The cut-off point for the size of myomas in terms of the occurrence of intraoperative hemorrhage was 61.5 mm; the sensitivity was 66.7% and specificity of 87.9% (Table 4).

**Table 4**  
Coordinates of the receiving operating characteristic (ROC) curve

Test result variable(s): myoma size	Sensitivity	1 - Specificity
Positive if greater than or equal to <sup>a</sup>		
9.0000	1.000	1.000
12.5000	0.972	1.000
16.5000	0.972	0.985
19.0000	0.972	0.970
21.5000	0.972	0.909
24.0000	0.972	0.894
26.5000	0.944	0.758
29.0000	0.944	0.742
31.0000	0.917	0.606
33.5000	0.917	0.591
36.5000	0.889	0.515
39.0000	0.861	0.515
42.5000	0.833	0.379
47.5000	0.806	0.318
52.5000	0.778	0.273
57.5000	0.722	0.242
61.5000	0.667	0.121
64.0000	0.667	0.106
67.5000	0.639	0.091
72.5000	0.556	0.076
77.5000	0.472	0.045
82.5000	0.417	0.045
87.5000	0.333	0.030
92.5000	0.306	0.000
97.5000	0.222	0.000
105.0000	0.194	0.000
115.0000	0.167	0.000
125.0000	0.139	0.000
140.0000	0.111	0.000
180.0000	0.028	0.000
211.0000	0.000	0.000

Intraoperative hemorrhage was registered in 75% of the patients with myomas bigger than 60 mm.

The indications for CS in the studied groups are listed in Table 5. The most common indication for CS in the study group was myoma *previa* (33.3%), while in the control group the most frequent indications were disproportion and other (nonobstetric) indications (18.2% each).

The characteristics of surgeries in both groups are shown in Table 6. The groups did not differ in relation to the experience of the surgeon, the incidence of emergency CS and the number of uterine incisions. In 8 of the patients CM was performed through low transverse cesarean incision. Duration of surgery was significantly different between the groups. The operation on the patients of the study group was significantly longer (on average, 14.53 minutes). None of the patients underwent postpartum hysterectomy and/or ligation of hypogastric arteries.

### Discussion

CM is advised only in cases where it is necessary for safe extraction of fetus and the performance and/or suture a low uterine segment (LUS) incision of the uterus<sup>9</sup>. Sometimes, CM is unavoidable, or represents an alternative to corporal CS<sup>10</sup>. The only absolute contraindication for CM is a significant risk of hemorrhage, particularly in cases with uterine hypotony<sup>11,12</sup>. An important factor in the decision making is myoma localization in relation to the large blood vessels, as enucleation of myomas in the proximity of uterine arteries significantly increases the risk of hemorrhage<sup>11,12</sup>. Indications for CM are not clearly defined neither in the literature nor in obstetrical textbooks<sup>2</sup>. According to Ortac et al.<sup>13</sup> CM indications include patient's desire, symptomatic and degenerative myoma and myoma that may lead to postoperative complications and adverse perinatal outcomes in subsequent pregnancies.

**Table 5**

Indications for cesarean section (CS)			
Indication for CS	Study group n (%)	Control group n (%)	Total n (%)
Disproportion	2 (5.6)	12 (18.2)	14 (13.7)
Previous CS	1 (2.8)	4 (6.1)	5 (4.9)
Previous myomectomy	5 (13.9)	2 (3.0)	7 (6.9)
Hypertension	4 (11.1)	5 (7.6)	9 (8.8)
Fetal malpresentation	3 (8.3)	11 (16.7)	14 (13.7)
Prolonged first stage of labor	0 (0.0)	4 (6.1)	4 (3.9)
Myoma <i>previa</i>	12 (33.3)	5 (7.6)	17 (16.7)
Fetal indications	4 (11.1)	11 (16.7)	15 (14.7)
Other (nonobstetric) indications	5 (13.9)	12 (18.2)	17 (16.7)
Total	36 (100.0)	66 (100.0)	102 (100.0)

Study group – patients with intraoperative hemorrhage;

Control group – patients without intraoperative hemorrhage.

**Table 6**

Surgery characteristics of cesarean section (CS) delivery patients			
Characteristic	Study group	Control group	<i>p</i>
Duration of surgery (min)	77.64 ± 22.54	63.11 ± 16.43	0.000
Surgeons years of practice (year)	18.19 ± 7.03	16.02 ± 6.26	0.111
Number of incisions	2.25 ± 0.69	2.10 ± 0.75	0.342
Incidence of emergency CS (n)	12	27	0.452

Data are expressed as mean ± standard deviation unless otherwise indicated.

Study group – patients with intraoperative hemorrhage;

Control group – patients without intraoperative hemorrhage.

Similar views are presented by other authors<sup>2,14</sup>. Most authors agree that myomas localized in the area of LUS incision should be removed if possible, without additional incision in the uterus<sup>4-6,9</sup>, as we did in 8 of the patients. On the contrary, there are attitudes that all visible myomas should routinely be removed during CS<sup>4,15</sup>.

The most important reason for controversies regarding CM is the risk of intraoperative hemorrhage. Myomectomy is associated with significant risk of intraoperative hemorrhage, even outside of pregnancy, and this is further pronounced in pregnancy due to increased vascularization gravid uterus<sup>1,16,17</sup>. Also, the most common complication of the CS itself is bleeding and/or blood transfusion, with an incidence of 8.6%<sup>18</sup>. Studies on massive hemorrhage, postpartum hysterectomy, and even death due to hemorrhagic shock after CM mainly do not address the issue of CM<sup>3,19</sup>.

There is a difference in the incidence of intraoperative hemorrhage between our study, with the frequency of 35.29%, compared to the data of other authors<sup>5</sup>. Our investigation demonstrated a higher incidence of intraoperative hemorrhage during CM. There are several explanations for such a difference. One of those is the number of patients included. In some studies, the number of patients was several times smaller than in our study<sup>14-16,20</sup>. In the study of Burton et al.<sup>20</sup>, out of 13 patients who underwent CM, one (7.69%) exhibited intraoperative hemorrhage that resolved after uterine artery ligation and transfusion of a single dose of packed red blood cells. There are publications indicating the incidence of intraoperative hemorrhage even lower than that observed in the general population after CS<sup>21</sup>. Another possible cause of the difference is a selection bias, related to the type and size of the fibroids. According to our results, the type and size of fibroids are the most important factors that influence the occurrence of intraoperative hemorrhage. Kaymak et al.<sup>22</sup> documented the incidence of intraoperative hemorrhage of 12.5%. Multiple myomas in this study were present in only 10 patients. The incidence of intraoperative hemorrhage of 12.6% was observed by Roman and Tabsh<sup>23</sup> in women with fibroids the average diameter of 35 mm, out of which 18% were multiple and 23% pedunculated. Hassiakos et al.<sup>11</sup> reported the incidence of 10% in a study with also lower incidence of multiple fibroids.

The undoubted cause of the observed differences is the difference in type of anesthesia used for CS. Intraoperative hemorrhage during CS is pronounced in women operated in general anesthesia<sup>18</sup>. The observed patients were operated on under general anesthesia, which is one of the drawbacks of the presented research. Skjeldestad and Øian<sup>24</sup> demonstrated that CS under general anesthesia is associated with two times higher risk of excessive bleeding compared to CS under regional anesthesia. The reason for this is the relaxation of muscles of the uterus caused by anesthetics. In contrast to our study, some researchers have used a tourniquet following the extraction of the fetus in order to reduce intraoperative blood loss, as well as various methods ligation of the blood vessels for devascularization of the operative field<sup>5,21</sup>. The literature describes numerous surgical approaches to reduction of intraoperative hemorrhage during CM, but the data on their impact on the outcome of future pregnancies are scarce. Some

of these techniques are recommended only for patients who no longer want to have children, but are interested in the preservation of the uterus. Our preliminary experience in this field indicates that application of the intraoperative blood salvage could be the method of choice for avoiding allogeneic blood transfusion during CM, without jeopardizing future fertility<sup>25</sup>.

The difference between the groups in terms of the type and size of fibroids supports the significant impact of these myoma characteristics on the occurrence of intraoperative hemorrhage. Most patients in the study group had multiple myomas, whereas in the control group subserous myomas were most common. Intraoperative hemorrhage is more frequent with multiple myomectomy, even outside of pregnancy<sup>26</sup>. Most authors agree that the pedunculated and subserous myomas are those which can be safely enucleated during CS<sup>5,23</sup>, as supported by our results.

The size of myomas had an effect on the occurrence of intraoperative bleeding. The risk of hemorrhage was pronounced in women with fibroids larger than 60 mm. Intraoperative hemorrhage was registered in 75% of patients with myomas bigger than 60 mm. Roman and Tabsh<sup>23</sup> registered hemorrhage in 10.9% of women with myomas  $\geq 30$  mm diameter and  $< 60$  mm, and in 22.7% of the women with myomas bigger than 60 mm. The influence of the size of myomas on the occurrence of intraoperative hemorrhage during myomectomy outside the pregnancy is well known. Bleeding occurs during myoma enucleation and suturing the uterine defect. Thus the size of the defect resulting from myoma enucleation and speed of suturing it have a significant influence on the occurrence of intraoperative hemorrhage. In our study, operation on the patients of the study group lasted 14.53 minutes longer and their myomas were 39 mm bigger, with no difference in surgical experience of the obstetricians. According to Fok et al.<sup>27</sup>, surgical experience affect both the duration of CS and intraoperative blood loss. They suggested that experienced surgeons sutured operative wounds more quickly, that shortened the surgery and made the intraoperative blood loss less. This conclusion cannot be fully applied to CM, since in these cases the time required to establish hemostasis, and thus the duration of operation, are mainly affected by the characteristics of the removed myoma, primarily its size. For the establishment of full uterine contractility, and thus stopping bleeding, it is necessary to establish its anatomical integrity. This may explain why surgical experience did not significantly affect the incidence of intraoperative hemorrhage in our study. Furthermore, in some cases, younger doctors operated with more experienced surgeons. Maybe, in some cases CS was done by less experienced obstetricians, and CM by their experienced first assistants, while that in cases of intraoperative hemorrhage, experienced surgeons took over the operation. In both instances, the operative notes recorded younger doctors as leading surgeons. All these are the reasons why surgical experience did not affect the occurrence of intraoperative hemorrhage in our research. Similar results were found by Bergholt et al.<sup>28</sup>, who did not find a relationship between the experience of the surgeon and intraoperative blood loss during CS in a teaching hospital.

Uncontrolled hemorrhage, the most severe complication of this procedure, which may lead to postpartum hysterectomy, was not registered in the presented research, despite the differences between our study and research published by other authors, as seen through the incidence of intraoperative hemorrhage. Also, none of the patients in our study required hypogastric artery ligation. According to Exacoustos and Rosati<sup>3</sup>, out of nine patients who underwent CM, three required hysterectomy due to massive hemorrhage. On the contrary, there are many studies about CM without cases of postpartum hysterectomy<sup>6,9,21,22</sup>. Those describing the cases of massive hemorrhage and consequent postpartum hysterectomies are rare and the number of cases published in the literature is probably less than what is really in practice<sup>1,3</sup>.

This study has some limitations. The main limitations of this study are retrospective design and small number of patients. In addition, this series provide the experience of a single teaching hospital. Possible influence of myoma localization, indications and types of CS, the number of incisions on the uterus and the neonatal birth weight on the risk of intraoperative hemorrhage would require testing on a bigger sample size. The lack of statistical significance in the presented study does not yet mean that these factors have no influence on the inci-

dence of intraoperative hemorrhage. This investigation, analyzing the risk factors for intraoperative hemorrhage concluded that CM is associated with a substantial risk of hemorrhage. The most important factors that contribute to this are myoma type and size, in cases of multiple and intramural myomas, bigger myomas and prolonged surgeries.

CM was promoted in many studies, without any serious or life-threatening complications in the presence of experienced surgeons. Nevertheless, even if we have the benefit of two operations in just one surgery, clinicians had to consider a possible risk for intraoperative hemorrhage.

### Conclusion

This study provides valuable information on concealing the women seeking CM. Interval myomectomy might represent a safer option in some of those patients. Furthermore, it would be advisable to discuss hemorrhage and transfusion risks with patients with big multiple and intramural myomas before making decision to perform CM. We hope that the presented results could be useful to obstetricians while deciding when to perform CM. Those who decide to perform CM should be prepared to manage intraoperative hemorrhage during surgery in cases they encounter it.

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## Prevalence of overweight and obesity in professional soldiers of the Czech Army over an 11-year period

Prevalencija prekomerne telesne mase i gojaznosti kod profesionalnih vojnika Vojske Češke u 11-godišnjem periodu

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### Abstract

**Background/Aim.** Obesity is currently considered to be the most frequent metabolic disease worldwide, not only in developed but also in developing countries. The aim of this work was to describe the development of health status in soldiers of the Armed Forces of the Czech Republic (ACR) and to emphasize the markers of non-communicable diseases. Our study describes the anthropometric and biochemical parameters of a large group of Czech Army professional soldiers. Data were obtained over a period of 11 years. **Methods.** During the monitored period, from 1999 to 2009, military physicians carried out on the average 6,360 examinations on professional soldiers *per* year and monitored their health and nutritional status with the aim of preventing the risk factors of non-communicable diseases. These examinations are compulsory for all professional soldiers at the age of 25, 30, 33, and 36 years. From the age of 39, these examinations are carried out every year till the end of their career. Besides taking personal histories and carrying out standard physical examinations, blood was taken for biochemical examination. The following anthropometric parameters were monitored: body constitution using body mass index (BMI) and waist circumference. Our study describes only part of the data concerning anthropometric and biochemical parameters of professional soldiers which were obtained over a period of 11 years. **Results.** Average BMI values in men were in the overweight range (26.5–27 kg/m<sup>2</sup>). Average values of waist circumference, however, ranged from 91.9 cm to 93.4 cm. Between the first and the last year of monitoring a statistically significant decrease in these values ranging from 93.4 ± 9.8 cm to 92.7 ± 9.5 cm ( $p < 0.001$ ) was ob-

served. All monitored anthropometric parameters in female professional soldiers were within normal limits. During the monitored period the proportion of overweight men gradually increased from 52% to 57.1% ( $p < 0.001$ ). There were no statistically significant changes in the prevalence of obesity in men (12–15%). Average glycaemia levels were within normal range in both men and women. A statistically significant decrease in these levels, however, was observed in men (from 5.1 ± 0.9 mmol/L to 4.8 ± 0.7 mmol/L ( $p < 0.001$ )) and in women (from 4.9 ± 0.6 mmol/L to 4.6 ± 0.6 mmol/L ( $p < 0.001$ )). Concerning the lipid profile in men, a significant decrease in average values of total cholesterol, triglycerides and LDL cholesterol was observed; from 5.5 ± 1.1 mmol/L to 5.1 ± 1.0 mmol/L ( $p < 0.001$ ), from 2.0 ± 1.6 mmol/L to 1.6 ± 1.2 mmol/L ( $p < 0.001$ ) and from 3.4 ± 1.1 mmol/L to 3.2 ± 0.9 mmol/L ( $p < 0.001$ ), respectively. **Conclusion.** During the monitored period only one-third of military professionals had normal body weight. More favorable situation was in female professional soldiers, two-thirds of them had normal body weight during the monitored period. Additionally, the increase in the number of individuals with BMI values in the overweight range was observed. Although the number of overweight soldiers was overestimated as a result of the inclusion of individuals with increased body weight due to well-developed musculature, the number of overweight and obese soldiers is still high.

**Key words:** overweight; obesity; military personnel; prevalence; czech republic.

### Apstrakt

**Uvod/Cilj.** Gojaznost se smatra najučestalijim metaboličkim oboljenjem u svetu, ne samo u razvijenim, već i u zemljama u razvoju. Cilj ove studije bio je da se analizira stanje zdravlja vojnika oružanih snaga Republike Češke (*Armed Forces of the Czech Republic* – ACR) i da se ukaže na markere nezaraznih bolesti. Ova studija opisuje antropometrijske i biohemijske parametre velike grupe profe-

sionalnih vojnika Vojske Češke. Podaci su dobijeni tokom 11-godišnjeg perioda. **Metode.** Tokom navedenog perioda (1999–2009), vojni lekari vršili su prosečno 6 360 pregleda profesionalnih vojnika godišnje i pratili stanje njihovog zdravlja i uhranjenosti radi sprečavanja faktora rizika od nezaraznih bolesti. Ti pregledi su obavezni za sve profesionalne vojnike stare 25, 30, 33 i 36 godina. Od 39. godine starosti ti pregledi se vrše svake godine, do kraja karijere. Osim uzimanja lične anamneze i sprovođenja standardnih fi-

zikalnih pregleda, uzima se i krv za biohemijske analize. Kontrolisani su sledeći antropometrijski parametri: građa tela pomoću indeksa telesne mase (*body mass index* – BMI) i obima struka. Ova studija obuhvatila je samo jedan deo podataka o antropometrijskim i biohemijskim parametrima profesionalnih vojnika prikupljenih tokom 11 godina. **Rezultati.** Prosečne vrednosti BMI za muškarce bile su u opsegu prekomerne težine (26,5–27 kg/m<sup>2</sup>). Prosečne vrednosti za obim struka, međutim, bile su od 91,9 cm do 93,4 cm. Između prve i poslednje godine praćenja, statistički značajan pad ovih vrednosti kretao se od 93,4 ± 9,8 cm do 92,7 ± 9,5 cm ( $p < 0,001$ ). Svi posmatrani antropometrijski parametri kod ženskih profesionalnih vojnika bili su u granicama normale. U navedenom periodu proporcija gojaznih muškaraca postepeno je rasla od 52% do 57,1% ( $p < 0,001$ ). Nije bilo statistički značajne promene prevalencije gojaznosti muškaraca (12–15%). Prosečne glikemijske vrednosti bile su u opsegu normalnih i za muškarce i za žene. Statistički značajan pad tih nivoa, međutim, ustanovljen je kod muškaraca (od 5,1 ± 0,9 mmol/L do 4,8 ± 0,7 mmol/L) ( $p < 0,001$ ) i kod žena

(4,9 ± 0,6 mmol/L do 4,6 ± 0,6 mmol/L) ( $p < 0,001$ ). Što se tiče lipidnog profila muškaraca, zapažen je značajan pad prosečnih vrednosti ukupnog holesterola, triglicerida i LDL holesterola (od 5,5 ± 1,1 mmol/L do 5,1 ± 1,0 mmol/L) ( $p < 0,001$ ), od 2,0 ± 1,6 mmol/L do 1,6 ± 1,2 mmol/L) ( $p < 0,001$ ) i od 3,4 ± 1,1 mmol/L do 3,2 ± 0,9 mmol/L) ( $p < 0,001$ ), redom. **Zaključak.** Tokom perioda posmatranja samo je 1/3 vojnih profesionalaca imala normalnu telesnu masu. Mnogo povoljnija situacija bila je kod ženskih profesionalnih vojnika, te je 2/3 njih imalo normalnu telesnu masu tokom perioda praćenja. Osim toga, primećen je rast broja osoba sa vrednostima BMI izvan opsega prekomerne telesne masu. Iako je broj gojaznih vojnika precenjen kao posledica uključivanja osoba sa povećanom telesnom masom zbog dobro razvijene muskulature, broj prekomerno teških i gojaznih vojnika ipak je veliki.

**Ključne reči:**  
telesna masa, prekomerna; gojaznost; kadar, vojni; prevalenca; češka.

## Introduction

Obesity is currently considered to be the most frequent metabolic disease worldwide, not only in developed but also in developing countries. Not only adults are affected, but also more and more often children and teenagers. Despite continuous progress in treatment of obesity, the prevalence is constantly increasing<sup>1–3</sup>. It is, thus, necessary to carry out preventive and interventional procedures aimed not only at individuals, but also at specific groups in the population<sup>4</sup>.

The aim of this work was to describe the development of health status in soldiers of the Armed Forces of the Czech Republic (ACR). Our study describes anthropometric and biochemical parameters of a large group of Czech Army professional soldiers which were obtained over a period of 11 years. Before the introduction of the Programme of Extended Preventive Care into the Czech Army no valid data concerning overweight or obesity of soldiers were available. These data have been available only since 1999.

## Methods

The Programme of Extended Preventive Care was introduced into the Czech Army in 1999 and has been implemented every year since then<sup>5,6</sup>. The programme is focused on detecting risk factors in professional soldiers. Examinations are carried out by military physicians at the Medical Service Centers of catchment areas. The examinations are compulsory for all professional soldiers at the age of 25, 30, 33, and 36 years. From the age of 39, these examinations are carried out every year till the end of their career. Besides taking personal histories and carrying out standard physical examinations, blood is sampled for a biochemical examination of glucose, lipid and protein metabolism and liver enzymes. The following biochemical parameters are assessed: fasting glycaemia, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride levels. A venous blood sample is taken on an empty stomach and the above-mentioned parameters are de-

termined in certified laboratories using standard laboratory techniques. The following anthropometric parameters are monitored: body constitution using body mass index (BMI) and waist circumference. Attention is focused especially on the risks factors of metabolic diseases, overweight and obesity and the comorbidities resulting from them. Our study describes only part of the data concerning anthropometric and biochemical parameters of professional soldiers which were obtained over a period of 11 years. The obtained data are presented as an average, with a standard deviation, and a percentage representation of probands in individual categories of the above-mentioned parameters. An assessment of the first and the last year of monitoring was performed using a Student's *t*-test. In order to calculate the proportions of individuals in selected categories contingency tables were used followed by the  $\chi^2$  test for testing the difference in frequencies of the groups. A value  $\leq 0.05$  was considered as statistically significant. Statistical analysis of data was performed using the Statistica CZ 12 software (the StatSoft, USA).

## Results

During the monitored period, from 1999 to 2009, military physicians carried out on the average 6,360 examinations on professional soldiers *per* year. The number of soldiers examined in the individual years of the monitored period gradually increased. In 2009 over 8,000 men and women were examined. The number of examined women gradually tripled. In 2009 there were 975 female soldiers in all age categories. Between the first and the last year of monitoring a statistically significant increase in BMI values and body weight in men was observed. On the contrary, waist circumference decreased.

A statistically significant decrease in the average age of examined men was observed (40.1 to 38.1 years;  $p < 0.001$ ). On the contrary, a statistically significant increase in the average age of the examined women was observed (from 36.2 to 38.5 years;  $p < 0.001$ ) during the monitored period. A statistically significant increase in body height was seen in

both genders (from 178.1 cm to 179.8 cm in men and from 165.9 cm to 167.1 cm in women). A statistically significant increase in body weight (from 84.5 kg to 86.9 kg;  $p < 0.001$ ) was observed only in men. Average values of BMI and waist circumference in male and female professional soldiers are shown in Tables 1 and 2. Average BMI values in men were constantly in the overweight range between the years 1999 and 2009 (26.5–27 kg/m<sup>2</sup>). During the monitored period a slight increase in BMI was observed (from 26.6 ± 3.3 kg/m<sup>2</sup> to 26.9 ± 3.2 kg/m<sup>2</sup>; ( $p < 0.001$ )). On the contrary, average values of waist circumference in men ranged from 91.9 cm

to 93.4 cm. There was a statistically significant decrease in waist circumference between the first and the last year of monitoring (from 93.4 ± 9.8 cm to 92.7 ± 9.5 cm;  $p < 0.001$ ). The average BMI values in women ranged from 23.5 kg/m<sup>2</sup> to 25 kg/m<sup>2</sup>. During the monitored period no statistically significant change in these values was observed. The average values of waist circumference ranged from 75.4 cm to 78.5 cm. A slight increase in these values ranging from 77.4 cm to 78.2 cm was observed. However, this increase was not statistically significant. Figures 1–4 show frequencies of BMI and waist circumference categories in both men and women.

Table 1

Average values of separate parameters in men within 11 years					
Year	Age (years)	Height (cm)	Weight (kg)	Body mass index (kg/m <sup>2</sup> )	Waist circumference (cm)
1999 (n = 6154)	40.1 ± 8.1	178.1 ± 6.5	84.5 ± 11.9	26.6 ± 3.3	93.4 ± 9.8
2000 (n = 6404)	40.7 ± 8.1	177.0 ± 6.3	85.2 ± 11.9	26.3 ± 3.3	93.2 ± 9.9
2001 (n = 6161)	39.5 ± 8.4	178.0 ± 6.5	85.4 ± 12.0	26.8 ± 3.3	92.8 ± 9.9
2002 (n = 4995)	38.4 ± 8.1	178.6 ± 6.5	85.0 ± 12.0	26.6 ± 3.3	91.9 ± 10.0
2003 (n = 5306)	38.8 ± 7.9	178.9 ± 6.4	85.6 ± 12.0	26.7 ± 3.3	92.5 ± 10.0
2004 (n = 4056)	37.4 ± 8.0	179.1 ± 6.4	85.6 ± 11.9	26.7 ± 3.3	92.0 ± 9.8
2005 (n = 5021)	36.6 ± 7.6	179.4 ± 6.6	85.7 ± 12.1	26.3 ± 3.2	92.4 ± 9.8
2006 (n = 5419)	36.7 ± 7.6	179.5 ± 6.5	85.8 ± 12.1	26.6 ± 3.3	92.2 ± 9.7
2007 (n = 5786)	36.6 ± 7.3	179.4 ± 6.4	85.5 ± 11.6	26.5 ± 3.1	91.9 ± 9.2
2008 (n = 7057)	37.5 ± 7.4	179.7 ± 6.5	86.1 ± 11.8	26.7 ± 3.1	92.4 ± 9.4
2009 (n = 7496)	38.1 ± 7.2***	179.8 ± 6.5***	86.9 ± 11.8***	26.9 ± 3.2***	92.7 ± 9.5***

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Table 2

Average values of separate parameters in women within 11 years					
Years	Age (years)	Height (cm)	Weight (kg)	Body mass index (kg/m <sup>2</sup> )	Waist circumference (cm)
1999 (n = 330)	36.2 ± 7.2	165.9 ± 6.2	66.2 ± 12.2	24.0 ± 4.2	77.4 ± 10.9
2000 (n = 371)	36.7 ± 7.5	166.0 ± 6.1	67.9 ± 12.6	24.7 ± 4.5	78.5 ± 11.8
2001 (n = 431)	35.8 ± 7.0	166.6 ± 6.3	66.7 ± 11.4	24.0 ± 3.9	76.6 ± 10.7
2002 (n = 359)	35.1 ± 6.9	167.0 ± 6.2	65.7 ± 10.9	23.5 ± 3.6	75.4 ± 10.1
2003 (n = 524)	35.9 ± 7.1	167.0 ± 6.4	67.3 ± 12.1	24.1 ± 4.1	77.4 ± 11.1
2004 (n = 443)	34.8 ± 6.9	167.4 ± 6.5	66.5 ± 11.7	23.7 ± 3.9	76.2 ± 10.5
2005 (n = 540)	35.9 ± 7.0	167.0 ± 6.1	65.7 ± 11.0	23.6 ± 3.8	76.7 ± 9.8
2006 (n = 606)	35.9 ± 7.1	167.0 ± 6.2	66.6 ± 11.6	23.9 ± 3.9	77.6 ± 10.2
2007 (n = 671)	35.9 ± 7.1	167.2 ± 6.4	66.1 ± 10.8	23.6 ± 3.6	77.1 ± 9.9
2008 (n = 857)	37.5 ± 7.1	167.4 ± 6.3	67.2 ± 11.2	24.0 ± 3.7	77.5 ± 9.9
2009 (n = 975)	38.5 ± 6.8***	167.1 ± 6.2**	67.7 ± 11.8	24.2 ± 3.9	78.2 ± 10.1

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

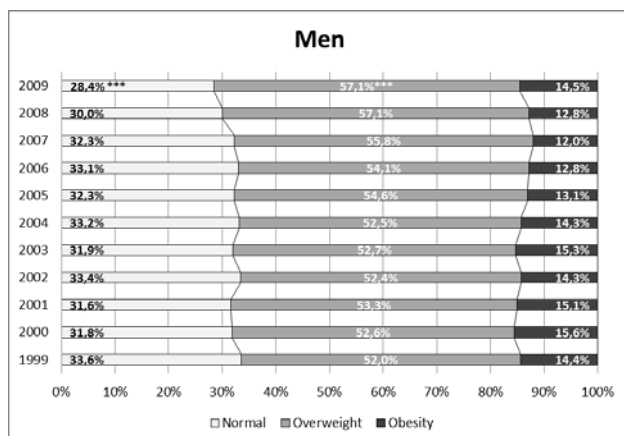


Fig. 1 – Distribution of men in the body mass index categories.

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

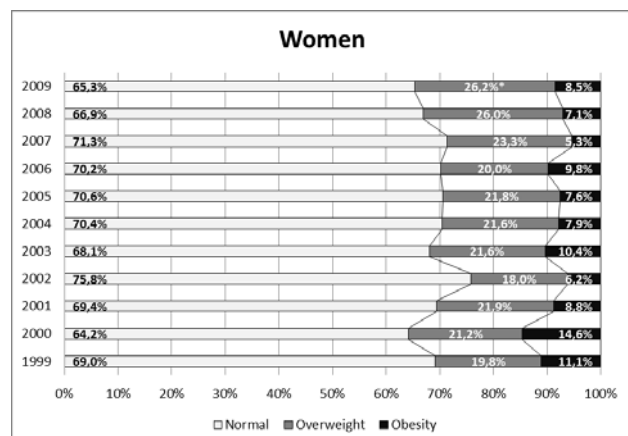
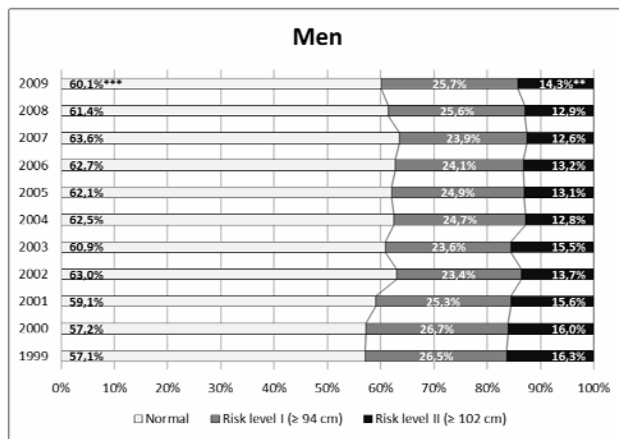


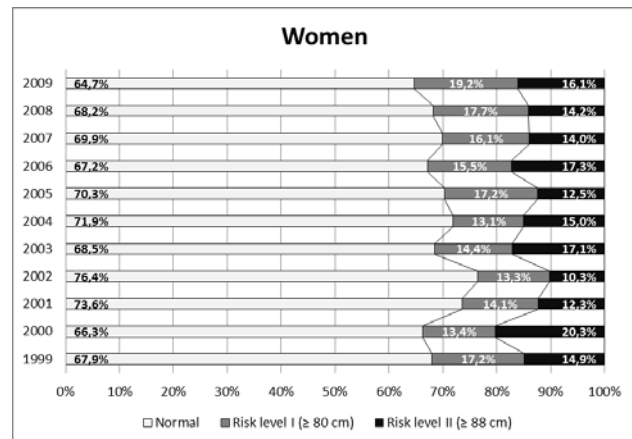
Fig. 2 – Distribution of women in the body mass index categories.

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Fig. 3 – Distribution of men in separate risk categories according to the waist circumference.**

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Fig. 4 – Distribution of women in separate risk categories according to the waist circumference.**

Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

The dynamics of frequencies of BMI values in men led to an increase in the number of probands with a higher BMI value. During the monitored period the proportion of overweight men gradually increased from 52% to 57.1% ( $p < 0.001$ ). There was no statistically significant change in the prevalence of obesity among men (12–15.6%). The same situation was seen in women. During the monitored period the number of female soldiers in whom the BMI values were found to be in the overweight range increased from 19.8% to 26.2% ( $p < 0.05$ ). The proportion of obese women varied ranging from 5% to 14%, however there was no significant trend. Metabolic risk was determined based on values of waist circumference. The men with a waist circumference  $\geq 94$  cm and the women with a waist circumference  $\geq 80$  cm (level I risk) were at moderate risk of developing metabolic disease. The men with a waist circum-

ference  $\geq 102$  cm and women with a waist circumference  $\geq 88$  cm were at high risk (level II risk)<sup>7, 8</sup>. In men, comparing the first and the last year of monitoring, a statistically significant decrease in individuals with higher values of waist circumference was observed (43–40%) ( $p < 0.001$ ). On the contrary, in women a slight statistically insignificant increase in individuals with higher values of waist circumference was seen (32–35%). The value of BMI in men was weakly associated with total cholesterol ( $r = 0.17$ ), LDL cholesterol ( $r = 0.17$ ), triglycerides ( $r = 0.28$ ) and glycaemia ( $r = 0.16$ ), all with statistical significance ( $p < 0.001$ ). A similar situation was in women, BMI was weakly associated with total cholesterol ( $r = 0.16$ ), LDL cholesterol ( $r = 0.22$ ), triglycerides ( $r = 0.25$ ) and glycaemia ( $r = 0.27$ ), all with statistical significance ( $p < 0.001$ ). Average values of biochemical parameters are shown in Tables 3 and 4.

**Table 3**

**Average values of biochemical parameters in men within 11 years**

Year	GLC (mmol/L)	TG (mmol/L)	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
1999 (n = 6,154)	5.1 ± 0.9	2.0 ± 1.6	5.5 ± 1.1	1.3 ± 0.4	3.4 ± 1.1
2000 (n = 6,404)	5.1 ± 0.9	2.0 ± 1.4	5.5 ± 1.1	1.3 ± 0.4	3.3 ± 1.1
2001 (n = 6,161)	4.9 ± 0.9	2.0 ± 1.5	5.4 ± 1.1	1.3 ± 0.4	3.4 ± 1.1
2002 (n = 4,995)	5.0 ± 0.9	1.8 ± 1.3	5.3 ± 1.1	1.3 ± 0.4	3.1 ± 1.0
2003 (n = 5,306)	4.9 ± 0.8	1.8 ± 1.4	5.3 ± 1.1	1.4 ± 0.4	3.2 ± 1.0
2004 (n = 4,056)	4.9 ± 0.8	1.8 ± 1.4	5.2 ± 1.1	1.4 ± 0.4	3.2 ± 0.9
2005 (n = 5,021)	4.9 ± 0.8	1.7 ± 1.4	5.2 ± 1.0	1.3 ± 0.4	3.2 ± 0.9
2006 (n = 5,419)	4.9 ± 0.8	1.7 ± 1.3	5.1 ± 1.0	1.3 ± 0.3	3.2 ± 0.9
2007 (n = 5,786)	4.8 ± 0.7	1.6 ± 1.3	5.1 ± 1.0	1.3 ± 0.4	3.3 ± 0.8
2008 (n = 7,057)	4.9 ± 0.7	1.7 ± 1.3	5.2 ± 1.0	1.3 ± 0.3	3.3 ± 0.9
2009 (n = 7,496)	4.8 ± 0.7 ***	1.6 ± 1.2 ***	5.1 ± 1.0 ***	1.3 ± 0.4 ***	3.2 ± 0.9 ***

GLC – glycaemia; TG – triglycerides; CHOL – total cholesterol; HDL – high density cholesterol; LDL – low density cholesterol; Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



Table 4

## Average values of biochemical parameters in women within 11 years

Year	GLC (mmol/L)	TG (mmol/L)	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
1999 (n = 330)	4.9 ± 0.6	1.3 ± 0.7	5.2 ± 1.0	1.6 ± 0.5	3.0 ± 1.0
2000 (n = 371)	4.7 ± 0.6	1.3 ± 0.8	5.2 ± 1.0	1.6 ± 0.4	2.9 ± 1.0
2001 (n = 431)	4.7 ± 0.9	1.2 ± 0.6	5.1 ± 1.0	1.6 ± 0.4	3.0 ± 0.9
2002 (n = 359)	4.7 ± 0.8	1.2 ± 0.6	5.0 ± 1.0	1.6 ± 0.4	2.8 ± 0.9
2003 (n = 524)	4.6 ± 0.6	1.2 ± 0.7	5.0 ± 0.9	1.6 ± 0.4	2.8 ± 0.8
2004 (n = 443)	4.6 ± 0.6	1.2 ± 0.6	5.0 ± 0.9	1.8 ± 0.4	2.8 ± 0.8
2005 (n = 540)	4.6 ± 0.7	1.2 ± 0.6	5.0 ± 0.9	1.7 ± 0.4	2.9 ± 0.8
2006 (n = 606)	4.7 ± 0.6	1.2 ± 0.7	4.9 ± 0.8	1.7 ± 0.4	2.8 ± 0.8
2007 (n = 671)	4.6 ± 0.6	1.1 ± 1.0	5.0 ± 0.9	1.7 ± 0.5	2.9 ± 0.7
2008 (n = 857)	4.7 ± 0.6	1.2 ± 0.6	5.1 ± 0.9	1.7 ± 0.4	3.0 ± 0.8
2009 (n = 975)	4.6 ± 0.6***	1.2 ± 0.6*	5.0 ± 0.9*	1.7 ± 0.4**	3.0 ± 0.8

GLC – glycaemia; TG – triglycerides; CHOL – total cholesterol, HDL – high density cholesterol, LDL – low density cholesterol; Statistical significance between the years 1999 and 2009 at the significance level – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Average glycaemia levels were in normal range in both men and women throughout the monitored period. However, a statistically significant decrease in these levels, from  $5.1 \pm 0.9$  mmol/L to  $4.8 \pm 0.7$  mmol/L ( $p < 0.001$ ) in men and from  $4.9 \pm 0.6$  mmol/L to  $4.6 \pm 0.6$  mmol/L ( $p < 0.001$ ) in women was seen. The percentage of men with the glycaemia levels in the range 5.6–6.9 mmol/L and above 7 mmol/L decreased, from 16% to 8.4% ( $p < 0.001$ ) and from 2.2% to 0.5% ( $p < 0.001$ ), respectively. Concerning the lipid profile in men, a significant decrease in average values of total cholesterol, triglycerides and LDL cholesterol was observed; from  $5.5 \pm 1.1$  mmol/L to  $5.1 \pm 1.0$  mmol/L ( $p < 0.001$ ), from  $2.0 \pm 1.6$  mmol/L to  $1.6 \pm 1.2$  mmol/L ( $p < 0.001$ ) and from  $3.4 \pm 1.1$  mmol/L to  $3.2 \pm 0.9$  mmol/L ( $p < 0.001$ ), respectively. The percentage of men with the levels of total cholesterol above 5 mmol/L decreased very significantly, from 66–51.2% ( $p < 0.001$ ). Similarly at the beginning of the monitored period there were 45.4% of men with triglyceride levels above 1.7 mmol/L. However, during the monitored period this percentage decreased to 32.9% ( $p < 0.001$ ). In women average levels of lipid profile also decreased, however not so significantly. On the contrary, a statistically significant decrease in the levels of total cholesterol and triglycerides was seen: from  $5.2 \pm 1.0$  mmol/L to  $5.0 \pm 0.9$  mmol/L ( $p < 0.05$ ) and from  $1.3 \pm 0.7$  mmol/L to  $1.2 \pm 0.6$  mmol/L ( $p < 0.05$ ), respectively.

### Discussion

The work provides information about anthropometric and biochemical parameters in professional soldiers of the ACR within 11 years. It also evaluates the importance of BMI as an independent marker for the diagnosis of overweight and obesity.

Other armies also struggle with overweight and obesity. Research performed in these armies has shown that the occurrence of overweight and obesity corresponds with the situation in general population. The situation in the Czech Army is not different.

Considering the length of monitoring and the number of examined probands in the ACR, there are not many studies

in the prevalence of obesity in large groups in other armies. Only the work by British authors describing the prevalence of overweight and obesity in more than 50,000 British soldiers is comparable<sup>9</sup>. According to this work, overweight occurs in 56% of soldiers and obesity in 12% of soldiers in the monitored group. The work of American authors deals with the prevalence of obesity in American soldiers during a long period of time, within which (between the years 1988 and 2004) body weight in the monitored group significantly increased by 2.4 kg<sup>10</sup>. Other American data present 50–54% of American soldiers in the range of overweight and obesity<sup>11</sup>. Data about adult American population present 34% of overweight and 34% of obesity<sup>12</sup>. The work by Polish authors present the prevalence of overweight and obesity in the Polish Armed Forces amounting to 14%<sup>13</sup>.

The above-mentioned retrospective data analysis concerning the health and nutritional status of professional soldiers shows that the average BMI values did not change significantly throughout the monitored period. In men they ranged from 26.5 kg/m<sup>2</sup> to 27 kg/m<sup>2</sup>, in women from 23.5 kg/m<sup>2</sup> to 25 kg/m<sup>2</sup>. On the contrary, the number of overweight soldiers gradually increased: from 52% to 57.1% ( $p < 0.001$ ) in men and from 19.8% to 26.2% ( $p < 0.05$ ) in women. The prevalence of obesity varied in both men and women, however without any significant trend. Obesity occurred in 12% to 15.6% of men and in 5% to 14% of women. One would assume that the occurrence of overweight soldiers would be significantly lower in the Czech Army than in the Czech population. However, even though the soldiers are a selected subpopulation, the results of research carried out among the Czech population repeatedly in the last 15 years show that this is not the case<sup>14–16</sup>. The number of obese soldiers is half the number of obese civilians. On the contrary, the number of overweight soldiers is twice as high as the number of overweight civilians.

When evaluating another anthropometric parameter, waist circumference, the number of men with increased values of this parameter, that means individuals with a risk of health complications, was significantly lower than according to the BMI criterion. Throughout the monitored period, a sta-

tistically significant decrease in with higher values of waist circumference was observed (from 43% to 40% ( $p < 0.001$ ). On the contrary, in women a slight statistically insignificant increase in individuals with higher values of waist circumference was seen (from 32% to 35%).

However, the body weight according to BMI increased in more than 66% of men and more than 31% of women. Especially in men there was a big disproportion between the calculated BMI and the measured waist circumference. The difference was observed only in individuals slightly exceeding the recommended values whose BMI was in the overweight range and who were at moderate risk of developing metabolic diseases. From 52% to 57% of men were overweight. However, a higher values of waist circumference in the range of moderate risk were observed only in 23–27% of men. When the values of BMI and waist circumference were in the range of obesity, both parameters had the same result. On the contrary, in female subpopulation with lower amount of muscular mass, both the BMI values and the values of waist circumference in percentage were the same.

The above-mentioned results confirm the assumption that also individuals with a normal amount of body fat and an increased muscular mass are ranged into the overweight category and in some cases into Class I obesity. A system evaluating the health capability of professional soldiers to carry out their military duties has been introduced into the Czech Army. One of the evaluation criteria of this system is the soldier's nutritional status assessed according to BMI values. Based on the current legislature BMI values in the range of 30–39.9 kg/m<sup>2</sup> mean that the health capability of soldiers is ranged into a lower classification and that prevents them from carrying out certain military duties (special force units, paratrooper units, participation in military missions abroad).

Long-term assessments of health condition of military professionals have shown that the BMI criterion itself is not sufficient, especially for evaluating overweight. Therefore, in the future it will be necessary to include the waist circumference and total body fat in the diagnostic criteria for obesity<sup>17–19</sup>. These two parameters can be additional parameters to BMI and can help identify individuals at risk of developing obesity and associated complications. The before-cited studies<sup>17–19</sup> prove on different groups of probands that using more anthropometric methods for determining total nutritional status of the organism is advantageous. The work of Belgian authors compares two anthropometric methodologies – BMI and impedance for determining total body fat in Belgian recruits<sup>20</sup>.

Despite seemingly high occurrence of overweight and obesity calculated using BMI, the number of individuals with increased values of biochemical parameters was significantly lower. During the monitored period there was an additional decrease in the values of the above-mentioned parameters. The most significant decrease was seen in glycaemia levels which were within normal range in both men and women. In most professional soldiers, both males and females, glycaemia levels were within normal range. The glycaemia levels in the range of prediabetes were observed in only 10% of monitored soldiers (fasting glycaemia above 5.6 mmol/L)<sup>21</sup>.

A return to normal levels of total cholesterol and triglycerides was observed in approximately 15% of men. The number of men with cholesterol levels within normal range increased from one-third to one-half. Similar situation was seen in triglycerides. At the beginning of the monitored period only half of male soldiers had normal triglyceride levels. However, the number gradually increased up to two-thirds which is a favorable trend, especially in prevention of cardiovascular diseases<sup>22,23</sup>.

### Conclusion

Military professionals are a selected subpopulation represented partly by young, physically fit individuals without any serious health problems. Thus, a high number of overweight and obese soldiers is very alarming. Throughout the monitored period only one third of military professionals had a normal body weight. Furthermore, the number of individuals with BMI values in the overweight range increased during this period. More favorable situation was in female professional soldiers, two-thirds of them had normal body weight during the monitoring period. However, it is necessary to take into consideration the limitations of BMI as a tool for evaluating the nutritional status, especially for the overweight category. The number of overweight soldiers was overestimated as a result of inclusion of individuals with increased body weight due to well-developed musculature. Therefore, in the future it will be necessary to include the waist circumference and total body fat in the diagnostic criteria for obesity used in evaluating health capability of professional soldiers.

Within the Czech Army we now have the opportunity to influence a large population group by providing primary prevention of non-communicable diseases. Using the Programme of Extended Preventive Care we can detect individuals at risk of developing non-communicable diseases early and begin targeted intervention.

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## Evaluating empathy in adolescents with conduct disorders

### Ispitivanje empatije kod adolescenata sa poremećajem ponašanja

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#### Abstract

**Background/Aim.** According to currently available data, there is no research dealing with evaluating empathy in adolescents with conduct disorders in our region. The aim of the research was to examine the differences in the severity of cognitive and affective empathy in adolescents with and without conduct disorder, as well as to examine the relationship between cognitive and affective empathy and the level of externalization in adolescents with conduct disorder. **Methods.** This research was conducted on 171 adolescents, aged 15 to 18, using the Interpersonal Reactivity Index, Youth Self-Report and a Questionnaire constructed for the purpose of this research. **Results.** The results showed that adolescents with conduct disorder had significantly lower scores for Perspective Taking ( $t = 3.255, p = 0.001$ ), Fantasy ( $t = 2.133, p = 0.034$ ) and Empathic Concern ( $t = 2.479, p = 0.014$ ) compared to the adolescents in the control group, while the values for Personal Distress ( $t = 1.818, p = 0.071$ ) were higher compared to the control group, but the difference was not statistically significant. The study showed a statistically significant negative correlation between Perspective Taking and aggression ( $r = -0.318, p = 0.003$ ) and a negative correlation between Perspective Taking and the overall level of externalizing problems ( $r = -0.310, p = 0.004$ ) in the group of adolescents with conduct disorder. **Conclusion.** This research contributes to better understanding of behavioral disorders in terms of individual factors, especially empathic reactivity. Preventive work with young people who have behavioral problems associated with empathy deficit disorder proved to be an important tool in preventing the development, or at least relieving the symptoms, of this ever more common disorder.

**Key words:** adolescents; conduct disorder; empathy; aggression; questionnaire.

#### Apstrakt

**Uvod/Cilj.** Prema trenutno raspoloživim podacima, u našoj sredini nema istraživanja empatije kod adolescenata sa poremećajem ponašanja. Cilj ovog istraživanja bio je da se ispituju razlike u izraženosti kognitivne i afektivne empatije između adolescenata sa i bez poremećaja ponašanja, kao i povezanost kognitivne i afektivne empatije i nivoa eksternalizacije kod adolescenata sa poremećajem ponašanja. **Metode.** Istraživanjem je obuhvaćen 171 ispitanik, uzrasta od 15 to 18 godina. Primenjeni su Indeks interpersonalne reaktivnosti, Upitnik za samoprocenu mladih od 11 do 18 godina i Opšti upitnik sačinjen za potrebe ovog istraživanja. **Rezultati.** Adolescenti sa poremećajem ponašanja postigli su statistički značajno niže vrednosti na dimenzijama kognitivne empatije *Perspective Taking* ( $t = 3,255, p = 0,001$ ), *Fantasy* ( $t = 2,133, p = 0,034$ ) i afektivnoj dimenziji *Empathic Concern* ( $t = 2,479, p = 0,014$ ) u odnosu na adolescente kontrolne grupe, i više vrednosti na dimenziji *Personal Distress* ( $t = 1,818, p = 0,071$ ) u odnosu na kontrolnu grupu, ali ne na nivou statističke značajnosti. U istraživanju je nađena statistički značajna negativna povezanost kognitivne dimenzije empatije *Perspective Taking* i agresije ( $r = -0,318, p = 0,003$ ), kao i negativna povezanost *Perspective Taking* i ukupnog nivoa eksternalizacionih problema ( $r = -0,310, p = 0,004$ ) u grupi adolescenata sa poremećajem ponašanja. **Zaključak.** Ovo istraživanje doprinosi boljem razumevanju poremećaja ponašanja sa aspekta individualnih faktora, pre svega empatijske reaktivnosti. Preventivni rad sa mladima koji imaju probleme ponašanja udružene sa nedostatkom empatije pokazao se kao značajno oruđe u sprečavanju razvoja ili bar ublažavanju simptoma ovog sve učestlijeg poremećaja.

**Ključne reči:** adolescenti; ponašanje, poremećaji; empatija; agresivnost; upitnici.

## Introduction

Empathy is defined as “an emotional reaction that stems from comprehension and apprehension of another person’s emotional experience or situation which is identical or similar to what the other person feels or should feel”<sup>1</sup> and represents the basis of social functioning and effective interaction in a social environment. Empathy appears early in life and its development is conditioned by individual factors (genetics, neural factors, temperament) and socialization factors (imitation, emotional quality of parenting, parent-child relations). Social outcomes of empathy affect the behavior towards others (internalization rules, prosocial behavior) as well as social relations (social competence, the quality of interpersonal relations). Davis<sup>2</sup> gave a rather broad definition of empathy and proposed that it was a “complex cognitive and affective response to the experiences of others”. The complex empathic response is multidimensional and involves cognitive (perspective taking, fantasy) and affective aspects (empathic concern, personal distress). The abilities for perspective taking and empathic concern increase from childhood to adolescence and reach adult levels, while personal distress decreases<sup>3</sup>. By the end of adolescence, a person is able to take a broader perspective and feel concern for other people, as well as analyze these aspects and act accordingly<sup>4</sup>. Many studies show the positive correlation between empathy and prosocial helping behavior in children and adolescents<sup>5</sup>.

The model of empathy that includes both cognitive and affective aspects involves several mechanisms which should lead to a reduction of aggression and increasing prosocial behavior in an empathetic person: the ability to discriminate and indicate the feelings of other people in social conflicts, a more mature cognitive ability which is responsible for perspective taking and should lead to conflict mitigation, as well as affective responsiveness, which has a special role in aggression regulation<sup>6</sup>. Compassion and better understanding of other people’s feelings and another person’s general condition make it possible for empathetic children to resolve conflicts successfully because their cognitive and emotional understanding of interpersonal situations inhibit aggressive reactions<sup>7</sup>.

Adolescents with conduct disorder often lack positive motivation and are not able to take another person’s perspective or take care of other people’s needs, understand the harmful effects of their actions on others and experience guilt<sup>8</sup>. There are only a few studies that have been performed on a clinical sample; therefore, little is known about the nature and causes of empathic dysfunction in adolescents with conduct disorder<sup>9</sup>. There is also not enough consistent data about which specific dimensions of empathy are disrupted in this disorder. As conduct disorder is becoming ever more common in our region, and has implications for both individual and social environment, it is important to test the hypothesis about reduced empathy reactivity in adolescents with conduct disorder on our sample.

The aim of the study was to investigate whether there is a statistically significant difference in the prominence of cognitive and affective empathy (perspective taking, phantasy,

empathic concern, personal distress) between adolescents with conduct disorder and adolescents without conduct disorder and investigate the correlation between cognitive and affective empathy and the level of externalizing problems in adolescents with conduct disorder.

## Methods

The study was conducted at the Department of Children and Adolescent Psychiatry, Mental Health Clinic, Clinical Center Niš, Serbia in 2012 and 2013. It included 171 adolescents, aged 15 to 18. The examined group consisted of 86 outpatient or hospitalized adolescents with conduct disorders. The diagnosis of conduct disorder was based on clinical interviews and existing criteria for conduct disorder<sup>10</sup>. The subjects with the following comorbid diagnoses were excluded from the study: attention deficit disorder and activity disorder, mental insufficiency under 80 on the basis of standard psychological tests, acute psychotic disorder and drug addiction. The group without conduct disorder (the control group) consisted of 85 high school students. Both groups were matched for sex, age and place of residence. Subjects and parents/caregivers gave informed consent to participate in the research.

The questionnaire designed for the purpose of this research consisted of questions about sociodemographic characteristics of the participants: gender, age, the number of household members, parents’ marital status, as well as the presence of parental mental illness. The questionnaire was filled out by the researcher based on the interviews with adolescents and parents and the data from the medical records or polyclinic records.

The Interpersonal Reactivity Index (IRI)<sup>2</sup> is a multidimensional scale composed of 28 self-report items designed to measure both cognitive and affective components of empathy. The subscales of the IRI were arrived at by factor analysis and consisted of four subscales *per* seven items each: Perspective Taking (PT), Fantasy Scale (FS), Empathic Concern (EC) and Personal Distress (PD). The Perspective Taking scale measures the tendency to take the psychological point of view of others. The Fantasy Scale measures the tendency to get caught up in fictional stories and imagine oneself in the same situations as fictional characters. The Empathic Concern scale measures sympathy and concern for others. The Personal Distress scale measures the type of feelings (anxiety, etc.) that get in the way of helping others. The participants were asked to report their agreement or disagreement with certain statements on the Likert Scale 1 (strongly disagree) to 5 (strongly agree). The minimum and maximum scores on each subscale was from 7 to 35, respectively. The higher scores indicate higher levels of cognitive or affective empathy. Employing a summation of the IRI subscale scores as an index of high or low empathy is not possible because the four subscales do not positively correlate, i.e. the increases in every subscale are not considered indicative of greater levels of empathy<sup>2</sup>.

The Youth Self-Report (YRS)<sup>11</sup> is a scale of emotional problems and behavior problems. The questionnaire has two parts: competence scale and the scale of problems with 112 items, which are grouped into eight syndrome scales. The

seventh and eighth scale referred to the group of externalizing problems – aggressive behavior (behavior aimed at drawing attention, passive aggressive and open aggressive behavior), and rule breaking behavior (morality aspect, violation of the legal norms, socially immature and maladapted behavior) that represent symptoms of behavioral disorders. The examinees were supposed to assess the extent to which they could relate to a particular problem on the Likert scale. Responses ranged from 0 (not true) to 2 (completely true).

The results of the study were statistically analyzed on the scales in relation to the study objective (the sum of scores on the seventh and eight syndrome scales).

All data are presented as mean and standard deviation, or percent frequency. Comparisons between groups were made by *t*-test, Mann-Whitney test or  $\chi^2$ -test. A *p* value < 0.05 was considered statistically significant. Statistical analyses were done with SPSS 16.0 for Windows.

## Results

Sociodemographic characteristics of adolescents with and without conduct disorders are shown in Table 1.

The analysis of the YRS questionnaire showed the expected differences between the groups which suggest that adolescents with conduct disorder have significantly higher scores for Rule Breaking Behavior, Aggressive Behavior and total Externalization in comparison to the control group (Table 2). YSR was not standardized for adolescents in our region, which is why it was not possible to compare the results we obtained to the standard values for our population.

The results indicated that there is a statistically significant difference in three of the four dimensions of empathy on IRI between adolescents with conduct disorders and the control group (Table 3). The analysis showed significantly lower scores on Perspective Taking in adolescents with conduct disorder in comparison to the control group. Fantasy was significantly lower in adolescents with conduct disorders in comparison to the control group. Empathic Concern was significantly lower in adolescents with conduct disorders in comparison to the control group. Personal Distress was higher in adolescents with conduct disorder in comparison to the control group, but the difference was not statistically significant (Table 3).

In the group of adolescents with conduct disorder, there was a correlation among all the dimensions of empathy,

**Table 1**  
Baseline characteristics in the two groups of adolescents

Parameter	Adolescents		<i>p</i>
	With conduct disorder (n = 86)	Controls (n = 85)	
Age (years), $\bar{x} \pm SD$	17.15 $\pm$ 0.97	17.19 $\pm$ 0.68	
Gender (M/F), n	43/43	40/45	0,817
The number of children in the family, n			0.008
1	15	12	
2	43	65	
> 2	21	8	
Divorced parents, n	28	8	< 0.001
Parental psychiatric disorders, n	36	9	0.001

$\bar{x}$  – mean values; SD – standard deviation.

**Table 2**

The average scores on the subscale for externalizing problems

Externalizing problems	Adolescents		<i>Z</i> *	<i>p</i>
	With conduct disorder (n = 86)	Controls (n = 85)		
Rule breaking behavior	11.31 $\pm$ 4.00 11.00 (4.00–26.00)	3.09 $\pm$ 2.41* 3.00 (0.00–11.00)#	10.717	< 0.001
Aggressive behavior	14.23 $\pm$ 5.19 14.00 (3.00–27.00)	6.28 $\pm$ 3.30 5.00 (0.00–17.00)	9.234	< 0.001
Externalization	25.54 $\pm$ 8.03 14.00 (3.00–27.00)	9.38 $\pm$ 4.91 5.00 (0.00–17.00)	10.548	< 0.001

\* – Mann-Whitney test. The values are presented as mean  $\pm$  standard deviation, median and (minimum-maximum).

**Table 3**

Differences in the dimensions of empathy in the adolescents with conduct disorders and the control group

Dimension of empathy	Adolescents		<i>t</i>	<i>p</i>
	With conduct disorder (n = 86)	Controls (n = 85)		
Perspective Taking	21.19 $\pm$ 4.47 20.00 (12.00–35.00)	23.49 $\pm$ 4.80 23.00 (12.00–35.00)	3.255	0.001
Fantasy	20.67 $\pm$ 5.23 20.00 (7.00–34.00)	22.41 $\pm$ 5.42 22.00 (11.00–35.00)	2.133	0.034
Empathic Concern	22.74 $\pm$ 3.84 23.00 (16.00–35.00)	24.28 $\pm$ 4.25 24.00 (14.00–33.00)	2.479	0.014
Personal Distress	20.05 $\pm$ 5.27 20.00 (7.00–33.00)	18.54 $\pm$ 5.55 19.00 (7.00–30.00)	1.818	0.071

The values are presented as mean  $\pm$  standard deviation, median and (minimum-maximum).

except Personal Distress. The correlation was positive and low. The highest correlation existed between Perspective Taking and Empathic Concern (Table 4). In the group of adolescents with conduct disorder, there was a statistically significant correlation among the scores on externalizing behavior. The correlation was positive and high. The highest correlation was between Aggressive Behavior and Externalization scores (Table 4). The analysis of the correlation between the scores on the dimensions of empathy and externalization scores showed a statistically significant negative correlation between Perspective Taking and Aggressive Behavior and Perspective Taking and Externalization scores (Table 4).

In the control group, there was a correlation among all the dimensions of empathy, except for Personal Distress. The correlation was positive and low. The correlation between Perspective Taking and Empathic Concern was strongest (Table 5). In the control group, there was a statistically significant correlation among the scores on externalizing behavior. The correlation was positive and high. The correlation between Aggressive Behavior and Externalization was strongest (Table 5). The analysis of the correlation between the scores on the dimensions of empathy and externalizing behavior showed a statistically significant negative correlation between scores on Perspective Taking and Rule Breaking Behavior, as well as Perspective Taking and Externalization scores (Table 5).

## Discussion

According to the literature which states that the presence or absence of individual, social and emotional responsiveness, which is associated with compassion, concern and understanding the other person's position, represent the protective or risk factors for the development of antisocial and aggressive behavior<sup>5</sup>, this research was based on the assumption that adolescents with conduct disorder exhibit reduced empathic reactivity compared to their peers without behavioral symptoms. Sociodemographic analysis of the data show that adolescents with conduct disorders often come from families that are characterized by many members, frequent divorce of parents and parents with multiple psychiatric disorders. These findings are consistent with previous studies<sup>12</sup>. Adolescents with conduct disorder had score significantly lower on Perspective Taking in comparison to the adolescents in the control group. Our results are consistent with the results of other studies<sup>13-15</sup>. The study by Lee and Prentice<sup>16</sup> shows that, compared to the control group, male delinquents have significantly lower scores not only on Perspective Taking, but also on cognitive tests and Kohlberg's moral dilemmas. Jolliffe and Farington<sup>17</sup> state that "offenders are ... insensitive and with low empathy. Their ability to take and understand others people's perspective is low and they may misinterpret other people's intentions. The lack of awareness

Table 4

### The correlation between the dimensions of empathy and externalization in the group of adolescents with conduct disorder

Scores	Fantasy	Empathic Concern	Personal Distress	Rule Breaking Behavior	Aggressive Behavior	Externalization
Perspective Taking	0.050	0.447**	0.128	-0.210	-0.318**	-0.310**
Fantasy	0.645	< 0.001	0.240	0.052	0.003	0.004
Empathic Concern	-	0.166	0.117	-0.068	-0.019	-0.046
Personal Distress		0.126	0.158	0.533	0.860	0.671
Rule Breaking Behavior		-	0.154	-0.206	-0.155	-0.203
Aggressive Behavior			0.076	0.057	0.153	0.061
Externalization			-	-0.066	0.089	0.025
				0.543	0.413	0.822
				-	0.517**	0.833**
					< 0.001	< 0.001

\* $p < 0.05$  (double); \*\* $p < 0.001$  (double).

Table 5

### Correlation between the dimensions of empathy and externalization in the control group

Scores	Fantasy	Empathic Concern	Personal Distress	Rule Breaking Behavior	Aggressive Behavior	Externalization
Perspective Taking	0.299**	0.439**	0.146	-0.347**	-0.195	-0.302**
Fantasy	0.005	< 0.001	0.182	0.001	0.073	0.005
Empathic Concern	-	0.429	0.120	-0.022	0.103	0.059
Personal Distress		< 0.001	0.154	0.840	0.347	0.594
Rule Breaking Behavior		-	0.159	-0.154	0.053	-0.040
Aggressive Behavior			0.072	0.160	0.632	0.717
Externalization			-	-0.103	0.023	-0.035
				0.350	0.836	0.750
				-	0.462**	0.802**
					< 0.001	< 0.001
					-	0.900**
						< 0.001

\* $p < 0.05$  (double); \*\* $p < 0.001$  (double).

or sensitivity to people's intentions and feelings diminishes their ability to assess the effects of their own behavior on others". The ability to discriminate and identify signs of other people's affection and take other people's perspective is a prerequisite for empathy which inhibits aggressive and antisocial behavior<sup>6</sup>. Fantasy is an essential factor in the cognitive aspect of empathy<sup>2</sup>, which means that imagination is one of the key factors that facilitate empathy and contribute to more vibrant experience of the observer, or the one who takes on this role. However, Fantasy is rarely used as a measure of interpersonal functioning in the available research data<sup>17</sup>. Our study shows that, compared to the control group, the adolescents with conduct disorder achieved statistically significantly lower scores on Fantasy. Cohen's study reported similar results<sup>13</sup>. Empathic Concern is a prosocial aspect of empathy. People who show higher scores on this dimension are characterized by greater emotional reactivity, vulnerability, and higher level of self-control<sup>2</sup>. It has been reported that people with greater empathic concern spend more time doing volunteering and helping the homeless, give more to charity and have a positive attitude towards the protection of animals<sup>18</sup>. The results based on the scores for Empathic Concern in our research support these findings<sup>13,15</sup> in the sense that adolescents with conduct disorder show significantly lower levels of compassion and concern for other people compared to the control group. Contrary to the expectations, our results show that adolescents with conduct disorder scored higher on Personal Distress than the adolescents in the control group, but the difference is not statistically significant. Cohen's<sup>13</sup> study reports that adolescents with conduct disorder have score significantly higher on Personal Distress and higher scores on this subscale correlate with greater aggression of the participants. The motivational role of personal distress in the process of empathy is most controversial in the literature. Batson<sup>19</sup> talks about personal distress as "the self-focused, aversive affective reaction that arises from the anticipation of another person's emotional experiences or state and is related to the desire to alleviate their own, not someone else's, distress. Personal distress is driven by a self-centered motivation to alleviate one's own stress<sup>1</sup>. On the other hand, other authors believe that individual differences in the level of personal distress or the ability to empathize are the result of general emotionality and the ability to regulate their own emotions<sup>20</sup>. Empathy underlies both of these processes, but the child who does not feel personal distress is able to act prosocially, whereas the child in personal distress is focused on him or herself and looks for ways to alleviate their own stress<sup>21</sup>.

The results of an Italian research on a larger sample of adolescents (142 females and 176 males, mean age 13.2 years) show that bullying among schoolchildren negatively correlate with the scores on Empathic Concern (affective dimension) and Perspective Taking (cognitive dimension) from the IRI questionnaire, and that helping the victimized peer actively positively correlates with the higher scores on the dimensions of empathy<sup>22</sup>. The research performed by Beven et al.<sup>23</sup> on a group of aggressive delinquents reported a negative correlation between the scores on Perspective Taking

and impulsivity and antisocial attitudes of the participants on the Criminal Sentiments Scale. The same research reported that the lower scores on Empathic Concern were associated with highly expressed antisocial attitudes and *vice versa*, pronounced Empathic Concern positively correlated to the prosocial attitudes of the participants<sup>23</sup>.

In the group of adolescents with conduct disorders, the results of the correlation analysis show that there is a negative correlation between Perspective Taking and aggression, and a negative correlation between Perspective Taking and the overall level of externalization. There were no significant correlation between other dimensions of empathy (EC, FS, PD) and aggression, rulebreaking behavior and the overall level of externalization. Based on the data from the literature, we expected a stronger negative correlation between the scores for the dimensions of empathy and externalization. One possible interpretation of our results concerns the questionnaire we used in our research (IRI), which divides empathy into several dimensions which can cause "poor" results, particularly on a smaller sample. Literature suggests that this is not the problem that is related only to the use of IRI; therefore, researchers believe that it is necessary to improve the instruments for measuring empathy since many of the currently available self-assessment instruments do not give expected results<sup>4</sup>. A negative correlation between Perspective Taking, aggression and the overall level of externalization in adolescents with conduct disorder indicates the importance of cognitive empathy for the appearance of externalizing problems. The ability to take on other people's perspective is a cognitive skill that promotes solving problems in a positive manner. Perspective Taking in conflict situations leads to better understanding of the other person's position, prevents destructive behavior and encourages prosocial actions<sup>6</sup>. The results of our research support the studies that report on the significant negative correlation between cognitive empathy and aggression<sup>17</sup>, rather than those which indicate a stronger inverse relationship between affective empathy and aggression and bullying<sup>24,25</sup>.

Analysis of the results regarding the correlation between the dimensions of empathy and externalization in the control group indicates that the lower scores on Perspective Taking correlate with the higher average scores on rule breaking behavior. Namely, the same dimension of empathy, Perspective Taking, correlates with externalizing problems both in the control group and the group of adolescents with conduct disorder. In the control group, there is a correlation with rule breaking behavior. Externalizing problems which were exhibited in the control group were expected. Almost every child sometimes violates social norms and the rights of other people or damages property, and almost all children go through a period of lying, stealing or playing truant<sup>26</sup>, which YRS questionnaire includes on the scale for rule breaking behavior. There was no significant correlation between other dimensions of empathy and externalizing problems, which can be explained by a vast interindividual variability in the sample of the control group.

It has been shown that empathy training may be a valuable tool to reduce aggression in school settings. There are



several empathy training techniques aimed at school children like Empathy Slide Series; training that include role playing, acting and storytelling. There is also Aggression Replacement Training which combines cognitive techniques with teaching of impulse control and moral reasoning<sup>27</sup>. The results indicated that the empathy training helped bring about more positive social behaviours and more positive self-evaluation in both aggressive and non-aggressive children. Pecukonis<sup>28</sup> has reported the usefulness of empathy training among 14–17-year old aggressive females in the residential treatment centre.

### Conclusion

This research shows that adolescents with conduct disorders have significantly lower scores on prosocial oriented di-

mensions of empathy – Perspective Taking and Empathic Concern, as well as on Fantasy on the Interpersonal Reactivity Index questionnaire, while the differences in Personal Distress are very close to the statistically significant values. In the group of adolescents with conduct disorder, there is a statistically significantly negative correlation between Perspective Taking and aggression and the overall level of externalization. The results of the research conducted for the purpose of this paper indicate the importance of individual factors, primarily empathic reactivity, for developing behavioral symptoms in youth. Preventive work which includes empathy training programs for young people who have behavioral problems associated with empathy deficit disorder proved to be an important tool in preventing the development, or at least relieving the symptoms, of this ever more common disorder among young people.

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## Long-term drug use and polypharmacy among the elderly population in the Republic of Srpska, Bosnia and Herzegovina

Stalna upotreba lekova i polifarmacija kod starije populacije u Republici Srpskoj, Bosna i Hercegovina

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### Abstract

**Background/Aim.** Prescription of drugs is a fundamental care component of the elderly. Elderly patients often take multiple drugs, and it is known that polypharmacy may lead to drug interactions and adverse events. The aim of this study was to analyze the long-term drug use and the prevalence of polypharmacy among the elderly population in the Republic of Srpska, Bosnia and Herzegovina. **Methods.** A retrospective study of outpatient drug use in 2005 and 2010 was conducted, analyzing prescriptions for patients aged  $\geq 65$  years reimbursed by the Health Insurance Fund. The study population was stratified by gender and age. Long-term drug use was defined as continuous drug dispensing for a whole year or at least two thirds of the year. Polypharmacy was defined as the use of 5 or more different reimbursed drugs. **Results.** Of all insured people aged  $\geq 65$  years, long-term drug use was identified in 10% (2005) and in 19% (2010), of whom 62% were women. Two to four different drugs were used by almost 49% (2005) and 54% (2010) of the elderly patients. The polypharmacy prevalence increased from 1.4% (2005) to 3.6% (2010); it increased in all the age groups of both genders. The largest increase was observed in the age group 65–74 years. Polypharmacy prevalence increase was higher in women. The most commonly used drugs were those for treatment of cardiovascular diseases, in particular drugs for hypertension and cardiac treatment. **Conclusion.** The study findings point out to the increase of elderly population with a long-term drug use. Over a half of elderly patients use 2–4 different drugs on the long-term basis. The polypharmacy prevalence was low. It increased in the period of 5 years in both genders. The increase was more prominent in women of all the age groups. The use of multiple drugs and polypharmacy increased with ageing.

### Key words:

drug, prescriptions; drug users; drug combinations; aged; bosnia-herzegovina.

### Apstrakt

**Uvod/Cilj.** Propisivanje lekova je ključna komponenta zdravstvene zaštite starijih osoba. Stariji često koriste više lekova, a poznato je da polifarmacija može dovesti do interakcija lekova i neželjenih događaja. Cilj ove studije bio je da se analizira stalna upotreba lekova i prevalencija polifarmacije u starijoj populaciji u Republici Srpskoj, Bosna i Hercegovina. **Metode.** Izvršeno je retrospektivno istraživanje upotrebe lekova koji se izdaju na recept u 2005. i 2010. godini, kroz analizu lekova propisanih osobama starim  $\geq 65$  godina koji se izdaju na teret Fonda zdravstvenog osiguranja. Ispitivana populacija bila je podeljena prema polu i starosti. Stalna upotreba leka definisana je kao neprekidno izdavanje leka tokom cele godine ili najmanje dve trećine godine. Polifarmacija definisana je kao upotreba pet i više različitih lekova koji se izdaju na teret obaveznog zdravstvenog osiguranja. **Rezultati.** Od svih osiguranika starosti  $\geq 65$  godina, stalna upotreba lekova koji se izdaju na teret zdravstvenog osiguranja utvrđena je kod 10% (2005) i 19% (2010) starijih, od čega su 62% bile žene. Dva do četiri različita leka koristilo je gotovo 49% (2005) i 54% (2010) starijih osiguranika. Prevalencija polifarmacije porasla je sa 1,4% (2005) na 3,6% (2010), i to u svim starosnim grupama oba pola. Najveći porast primećen je u starosnoj grupi 65–74 godine. Porast prevalencije polifarmacije bio je veći kod žena. Najčešće su korišćeni lekovi za lečenje kardiovaskularnih bolesti, naročito lekovi za lečenje hipertenzije i bolesti srca. **Zaključak.** Rezultati istraživanja ukazuju na porast starije populacije koja stalno koristi lekove. Više od polovine starijih osiguranika stalno koristi 2–4 različita leka. Prevalencija polifarmacije je niska. U 5-godišnjem periodu porasla je kod oba pola. Porast je izraženiji kod žena svih starosnih grupa. Upotreba više lekova i polifarmacija rasli su sa godinama osiguranika.

### Ključne reči:

lekovi, propisivanje; lekovi, korišćenje; lekovi, kombinacije; stare osobe; bosna i hercegovina.

## Introduction

Prescription of medicines is a fundamental care component of the elderly, thus optimization of drug prescribing for this group has become an important public-health issue. The increasing prevalence of polypharmacy in frail elderly population has already been demonstrated<sup>1,2</sup>. The risk of morbidity and mortality associated with polypharmacy, combined with the trend of population aging worldwide, makes polypharmacy an area of prime concern<sup>3</sup>.

Polypharmacy in the elderly has been described in several European countries<sup>2-4</sup>, and it has been correlated with increased age and female gender<sup>2,5</sup>. Bosnia and Herzegovina is characterized by the demographic transition, with an increase in life expectancy and an expressed aging of population. To our knowledge, the analyses of the use of drugs in the elderly population have so far not been performed in Bosnia and Herzegovina. Given the already identified prevalent use of polypharmacy in the elderly by other researchers<sup>6,7</sup> and aging of our population, we assumed that polypharmacy could also be present in our elderly population.

Drugs commonly used only for short terms are discontinued soon and patients are not likely to have adverse drug reactions from them. Therefore, the objective of this study was to analyze the long-term prescription drug use and the prevalence of polypharmacy in the elderly population.

## Methods

The study was performed in the Republic of Srpska, which is one of the two constitutive entities of Bosnia and Herzegovina with an estimated population of 1.4 million, and its own executive and legislative functional responsibilities including healthcare policies on its territory. The Health Insurance Fund (HIF) provides compulsory health insurance coverage for the population, including the list of reimbursed drugs which is based on Anatomical Therapeutic Chemical (ATC) classification<sup>8</sup>.

This was a retrospective study, analyzing all prescriptions for people aged  $\geq 65$  years reimbursed by HIF and dispensed by retail pharmacies during 2005 and 2010. These two-time windows were chosen as the first (2005) and the last (2010) year for which it was possible to get the data from the HIF's database on the same way, as the study was conducted in 2011. The following data from the database were used in this study: dispensed drug, dispensing date, age, gender and disease diagnose [according to the WHO International Classification of Diseases (ICD) revision 10]. All processing of the individual data of dispensed drugs in the study were undertaken anonymously, with a unique temporary individual identifier specifying gender and year of birth applied. The study population was stratified by gender and age into 10-year classes: 65–74 years, 75–84 years and  $\geq 85$  years. A list of reimbursed drugs was comprised of 130 and 203 different drugs given under international non-proprietary name (ATC level 5) in 2005 and 2010, respectively.

Long-term drug use was defined as continuous drug dispensing for a whole year or at least two thirds of the year<sup>9</sup>,

which implies drugs used for chronic diseases treatment. Drug use was assumed to start on the day the medication was dispensed. The number of different drugs used was stratified in three groups: 1, 2–4 and  $\geq 5$  drugs. Polypharmacy was defined as the use of five or more different reimbursed drugs – defined by the ATC-Code – during one year. This definition might enable comparisons with other studies as one of the most commonly used<sup>10-12</sup> because a consensus on the definition of polypharmacy is still lacking. The prevalence of drug use was defined as the proportion of elderly patients who used 1, 2–4 and  $\geq 5$  different reimbursed drugs during one year. Statistical analysis involved the overall statistical weight of the study population. Descriptive statistics were used to calculate prevalence proportions.

## Results

Of all insured people aged  $\geq 65$  years, long-term drug use was identified in 10% (19,403) and 19% (43,781) patients in 2005 and 2010, respectively, of whom 62% were women. Out of the total elderly with long-term drug use, patients aged 65–74 accounted 69% and 60%, 75–84 29% and 36% and  $\geq 85$  2% and 4% in 2005 and 2010, respectively. Of all prescriptions to the elderly, 29% (468,351) and 35% (954,135) were prescribed for long-term drug use in 2005 and 2010, respectively, and 60% of these were prescribed to women.

One drug alone was used by almost 50% and 43% of the elderly in 2005 and 2010, respectively, and it was used more by men of all the age groups, except the group 65–74 in 2005. The proportion of elderly who used one drug only decreased in all the age groups of both genders in 2010 (Table 1).

**Table 1**  
**Prevalence of the use of different drugs by gender and age (%)**

Age of patients (years)	Number of drugs used					
	2005			2010		
	1	2–4	$\geq 5$	1	2–4	$\geq 5$
All patients	49.8	48.8	1.4	42.9	53.6	3.6
65–74	50.9	47.4	1.2	44.1	52.6	3.3
75–84	47.4	50.8	1.9	41.3	54.8	3.9
$\geq 85$	44.2	53.3	2.6	38.9	56.7	4.5
Men	49.6	48.8	1.6	43.8	52.9	3.4
65–74	50.5	48.1	1.4	44.2	52.6	3.2
75–84	47.7	50.3	2.0	43.2	53.1	3.6
$\geq 85$	45.5	50.9	3.6	40.7	55.2	4.1
Women	49.9	48.8	1.3	42.3	54.0	3.7
65–74	51.2	47.7	1.1	43.9	52.7	3.4
75–84	47.2	51.1	1.8	40.1	55.8	4.1
$\geq 85$	43.6	53.4	2.1	37.9	57.4	4.7

Results are given as % of patients.

Two to four different drugs were used by almost 49% and 54% of the elderly in 2005 and 2010, respectively, and they were more used by women of all age groups, except the age group 65–74 in 2005. The proportion of patients who used 2–4 drugs increased in all age groups of both genders in 2010. With increasing age, the prevalence of use of 2–4 dif-

ferent drugs increased and only the prevalence of use of one drug decreased (Table 1).

The most commonly used drugs related to treatment of cardiovascular, metabolic, digestive and respiratory diseases (Table 2). Drugs for hypertension treatment, particularly angiotensin-converting enzyme inhibitors (ACEIs) and calcium channel blockers (CCB), and the drugs for cardiac therapy (digoxin, isosorbide mononitrate) were the most frequently used cardiovascular drugs. Drugs for diabetes and acid related disorders were the most used for treatment of metabolic and digestive diseases, and aminophylline for obstructive airway diseases (Table 3).

In the total observed elderly population polypharmacy prevalence increased from 1.4% in 2005 to 3.6% in 2010. It increased in all the age group, and the largest increase was observed in the age group 65–74. In men, polypharmacy prevalence increased from 1.6% to 3.4%, and in women, it increased from 1.3% to 3.7% in 2005 and 2010, respectively. It increased in all the age groups, with the largest increase in the age group 65–74 in men and  $\geq 85$  in women (Table 1).

## Discussion

The study findings point out to the increase of elderly population with long-term drug use. Over a 5-year period, the proportion of the elderly who have used one drug alone on a long-term basis declined, while the proportion of those who used multiple drugs increased. Polypharmacy prevalence increased in both men and women, and the increase was more prominent in women of all age groups. The use of multiple drugs and polypharmacy increased with ageing.

Different reasons may have contributed to the observed increase of drug use in the elderly. Numerous new drugs and new pharmaceutical and dosage forms, mostly for treatment of cardiovascular, metabolic, digestive, nervous and respiratory diseases, were included in the list of reimbursable drugs during the observed period. Broader therapeutic options enabled better affordability and coverage of patients, and better insight in the extent of drugs used by the elderly, as these drugs were available on the market although previously not reimbursed. Furthermore, ageing of our population was evident: in the last two decades the overall population decreased by 12% while the proportion of people aged  $\geq 80$  tripled; people aged  $\geq 65$  constituted 19% of the population<sup>13,14</sup>. Population aging is known as a main risk factor for the development of chronic diseases and multiple drug use<sup>6</sup>. Diabetes, heart disease, hypertension and obstructive pulmonary disease are well-known relevant morbidity-related predictors of polypharmacy<sup>9,15</sup>, and all of them were shown as prevalent among our elderly. Also, a number of new clinical guidelines have been applied since 2004 that have certainly influenced physicians' prescribing patterns, as guidelines often recommend the use of several drugs to treat or prevent a disease. Prescription of multiple drugs may itself lead to the need for additional medication, e.g. ranitidine prescription due to the adverse gastrointestinal effects from drug intake.

The prevalence of polypharmacy in our study population was low. When defined as the use of five or more drugs, some previous studies reported that polypharmacy occurred in 4% and

29% of elderly patients with long-term drug use<sup>9,16</sup>. In order to compare the data with neighboring countries and according the available literature, polypharmacy was documented in about 10% elderly in Belgrade, Serbia<sup>12</sup>, while the other researchers focused more on the analysis of potentially inappropriate drug prescribing in the elderly<sup>17–20</sup>. Different results and limited comparability can be explained by differing inclusion criteria between studies, variations in duration of drug use and the research period, data collecting method and the representativeness of the study population. Considering the study methodology, our study results are similar to some previous findings<sup>9</sup>. Although low, polypharmacy prevalence increased during the period of five years. The development of chronic polypharmacy in long-term drug use is a slow process and the elderly who use more than four long-term drugs simultaneously are more likely to add another drug in a short time than those who use fewer than four long-term drugs<sup>9</sup>. According to the study findings, over a half of our elderly patients are already using 2–4 different drugs on the long-term basis for treatment of chronic diseases. Therefore, a special prescribing attention should be in particular focused to these patients, as the number of long-term drugs which a patient already uses is the best predictor of polypharmacy<sup>9</sup>.

More men of almost all age groups used one drug only both years, while more women used multiple drugs, except in 2005 when polypharmacy was more prominent in men. Many studies have reported a correlation between polypharmacy and female gender<sup>7,15,21,22</sup>, and a positive correlation between polypharmacy and male gender were found only in two<sup>3,23</sup>. Such discrepancies among study findings could be due to differences in physicians' prescribing attitude toward genders, as well as to differences between genders in educational and socioeconomic characteristics<sup>3</sup>. It is known that women consult health services more often and earlier than men, and are more accustomed to the use of drugs<sup>6</sup>. As in the total population, women had a higher share in the elderly insured population and they were prescribed by more prescriptions. Also, those who report poor self-perceived health status are most likely to take medications<sup>24</sup>, and in 2010 more women (20%) than men (16%) rated their health as worse than 12 months ago<sup>14</sup>. Further research exploring the relationship between gender and polypharmacy is needed.

The prevalence of polypharmacy, as well as the use of 2–4 different drugs increased in all age groups, and it displayed a clear age trend. Along with aging and increased availability of drugs, new national guidelines might also contribute to explaining the age trend in the development of polypharmacy. Older patients are more often exposed to several diseases, and they may receive, as a result of the guidelines, more often than others, an increasing number of different drugs<sup>11</sup>. The results of this study may also be interpreted to imply that a larger proportion of patients are receiving recommended drug treatment in line with the new guidelines, but further research is needed to clarify this issue as well.

Similar to other studies, our elderly mostly used drugs for cardiovascular, alimentary tract and metabolism and respiratory system diseases<sup>6,25</sup>. These similarities in consumption patterns may reflect common therapeutic needs among elderly patients, and applying a standard prescription scheme regarding patient's age<sup>26</sup>. High use of cardiovascular drugs is

**Table 2**  
**Most common diseases for which elderly were prescribed drugs (%)**

Disease (ICD-10)	2005				2010			
	Men (n = 7,561)	Women (n = 11,842)	Total (n = 19,403)	Frequency ranking	Men (n = 16,792)	Women (n = 26,989)	Total (n = 43,781)	Frequency ranking
I10 Hypertension	72.1	77.6	75.5	1	72.7	80.2	77.4	1
I20 Angina pectoris	34.7	31.5	32.7	2	24.0	22.7	23.2	2
I42 Cardiomyopathy	30.0	28.7	29.2	3	15.9	17.3	16.8	3
E11 Diabetes mellitus, type 2	10.9	13.3	12.4	4	14.3	17.0	16.0	4
E10 Diabetes mellitus, type 1	9.2	12.2	11.0	5	8.0	9.2	8.7	5
I50 Heart failure	2.3	2.3	2.3	14	8.5	9.0	8.8	6
K29 Gastritis and duodenitis	6.7	7.1	6.9	7	4.9	6.0	5.6	7
I25 Chronic ischaemic heart disease	6.9	5.7	6.2	8	6.0	5.1	5.5	8
I49 Cardiac arrhythmias	5.5	5.5	5.5	9	3.9	4.1	4.0	9
J42 Chronic bronchitis	11.2	6.1	8.1	6	5.8	3.7	4.5	10
J44 Other COPD	5.4	2.5	3.6	11	4.7	2.3	3.0	11
J45 Asthma	7.7	3.3	5.0	10	4.1	2.3	3.0	12
F32 Depressive episode	1.5	1.8	1.7	16	2.3	3.1	2.8	13
F20 Schizophrenia	2.5	2.8	2.7	15	1.1	1.3	1.2	14
F48 Other neurotic disorders	3.0	3.8	3.5	12	0.9	1.2	1.1	15
M54 Dorsalgia	2.2	2.9	2.6	13	0.6	0.9	0.8	16

ICD – International Classification of Diseases, 10<sup>th</sup> revision; COPD – chronic obstructive pulmonary disease.

**Table 3**  
**Most frequently prescribed drugs (%)**

ATC-Code	INN	2005				2010			
		Men (n = 7,561)	Women (n = 11,842)	Total (n = 19,403)	Frequency ranking	Men (n = 16,792)	Women (n = 26,989)	Total (n = 43,781)	Frequency ranking
C09BA02	Enalapril and diuretics	4.6	5.7	5.3	10	18.1	22.5	20.8	1
C09AA02	Enalapril	13.5	14.6	14.2	4	18.0	18.2	18.1	2
C08CA01	Amlodipine	17.4	18.4	18.0	2	14.9	16.7	16.0	3
C01DA14	Isosorbide mononitrate	19.8	16.2	17.6	3	13.7	12.8	13.2	4
C01AA05	Digoxin	10.0	10.9	10.5	5	9.6	10.6	10.3	5
A10BA01	Metformin	3.3	4.7	4.1	12	8.0	10.1	9.3	6
C09BA06	Quinapril and diuretics	1.9	2.8	2.4	19	5.4	7.3	6.6	7
C07AG02	Carvedilol	3.6	2.3	2.8	17	6.4	6.6	6.5	8
C07AB02	Metoprolol	na	na	na	na	4.9	6.7	6.0	9
A02BA02	Ranitidine	7.2	7.8	7.5	6	4.6	5.4	5.1	10
C08DA01	Verapamil	5.5	6.2	5.9	9	4.3	5.3	4.9	11
R03DA05	Amunophylline	9.1	4.4	6.2	8	6.1	3.4	4.4	12
C03CA01	Furosemide	na	na	na	na	4.1	4.0	4.1	13
A10AD05	Insulin aspart	na	na	na	na	3.5	4.2	3.9	14
S01ED01	Timolol	3.0	2.4	2.6	18	3.8	3.4	3.6	15
C10AA01	Simvastatin	1.8	1.4	1.6	20	3.6	2.8	3.1	16
C09AA06	Quinapril	3.0	3.5	3.3	14	3.0	3.2	3.1	17
A10BB09	Gliclazide	6.9	7.4	7.2	7	3.0	3.0	3.0	18
A10BB01	Glibenclamide	na	na	na	na	2.3	3.0	2.8	19
C08CA05	Nifedipine	4.2	3.9	4.0	13	2.5	2.5	2.5	20
N05BA01	Diazepam	4.6	5.4	5.1	11	2.1	2.4	2.3	21
C09AA01	Captopril	18.6	19.7	19.3	1	2.1	2.2	2.2	22
R03AC02	Salbutamol, aerosol	5.5	1.9	3.3	15	3.3	1.5	2.2	23
M01AE01	Ibuprofen	2.8	3.4	3.2	16	1.3	1.5	1.4	24

ATC – Anatomical Therapeutic Chemical; INN – International non-proprietary name; na – not applicable.

not a surprising finding, as cardiovascular diseases are a leading cause of morbidity and mortality in the population of the Republic of Srpska in the last decade<sup>27</sup>. As part of the national cardiovascular program a particular focus was put on the development of clinical guidelines<sup>28</sup> and a selection of cardiovascular drugs reimbursed by the HIF. New diuretics (furosemide, torasemide, spironolactone, combinations with amiloride), beta-blockers (metoprolol, bisoprolol), antiarrhythmics (amiodarone) and angiotensin-converting enzyme inhibitors (ACEIs) (ramipril, trandolapril) were included to the list of reimbursed drugs in 2010, with a tougher prescribing restrictions related to the dose, indications and copayment.

ACEI were the most prescribed antihypertensive drugs as they can be considered the first-line or combination therapy, especially if diabetes or heart failure is present. A high proportion of patients who used fixed-dosage combinations of ACEI with hydrochlorothiazide may be due to the guidelines indicating a strong preference for thiazide diuretic when combination therapies are needed, often for high risk patients<sup>28,29</sup>, and when lower doses may be used resulting in fewer side-effects and better compliance and adherence to prescribed antihypertensive drugs. The use of captopril decreased due to a broader availability of drugs dosed once-daily and a higher patient copayment (50%).

Calcium channel blockers (CCBs) are among the preferred for hypertension treatment in the elderly<sup>28</sup>, and amlodipine was widely prescribed as a safe drug for use in patients with heart failure, hypertension, chronic stable angina and diabetes<sup>30</sup>, which were all prevalent in our elderly. Among all available beta-blockers, carvedilol was probably more widely used in treatment of cardiovascular patients with metabolic syndrome or diabetes as it affect insulin sensitivity less than metoprolol<sup>31</sup>, and cardioselective metoprolol has a preferable side effect profile in older persons and a lower price than bisoprolol. Digoxin is traditionally overprescribed though the indications for this drug are sparse. This drug has been used in one out of ten patients on the long-term basis. According to cardiovascular guidelines, digoxin is no more the first line treatment for heart failure, but remains a useful drug for heart failure associated with atrial fibrillation. It is an inexpensive, but potentially risky drug for elderly patients due to its problematic safety profile associated with numerous side effects and potentially serious drug interactions.

Current guidelines for diabetes recommend early initiation of metformin as the first-line drug for monotherapy and

combination therapy for patients with type 2 diabetes. This was based primarily on metformin's glucose-lowering effects, relatively low cost, and generally low level of side effects<sup>32</sup>. The data of this study clearly showed that aminophylline is still a more frequently prescribed drug for treatment of chronic obstructive pulmonary disease (COPD) of our patients than any other bronchodilator. This was quite unexpected since in the major guidelines for treatment of COPD, theophylline and its derivate aminophylline are relegated to a third line bronchodilator after inhaled anticholinergics and  $\beta_2$ -agonists. Theophylline has narrow therapeutic index with potentially serious side effects, and theophylline clearance is significantly reduced in elderly patients. It is promising that the use of some traditional drugs, like ranitidine, gliclazide, captopril, nifedipine, ibuprofen and diazepam significantly decreased over the period of five years as a direct consequence of the introduction of new clinical guidelines into clinical practice.

The major advantage of this study is the large and reliable data set analyzed, covering all reimbursed prescriptions to every individual aged  $\geq 65$ . As all insured elderly were included, certain known problems concerning sampling, interview and confidence were avoided. The use of drugs may be underestimated as this database does not include prescription drugs not reimbursed by the HIF and non-prescription drugs. We do not know whether or not all analyzed drugs were actually used. It was not possible to determine the appropriateness of any drugs used.

## Conclusion

The study findings point out to an increase of elderly population with a long-term drug use. Over a half of our elderly patients use 2–4 different drugs on the long-term basis. The prevalence of polypharmacy was low, but it increased in the period of 5 years. This increase, observed in both genders, was more prominent in women of all the age groups. The use of multiple drugs and polypharmacy increased with ageing. Our elderly patients should be carefully monitored in terms of quality of care, patient safety, and costs of treatment.

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## Epidemiology and structure of congenital anomalies of the newborns in the region of Novi Sad (Vojvodina, Serbia) in 1996 and 2006

Epidemiologija i struktura kongenitalnih anomalija novorođenčadi u regionu Novog Sada (Vojvodina, Srbija) u 1996. i 2006.

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### Abstract

**Background/Aim.** According to the World Health Organization (WHO) definition, congenital anomalies are all disorders of the organs or tissues, regardless of whether they are visible at birth or manifest in life, and are registered in the International Classification of Diseases. The aim of this study was to compare the incidence and structure of prenatally detected and clinically manifested congenital anomalies in the newborns in the region of Novi Sad (Province of Vojvodina, Serbia) in the two distant years (1996 and 2006). **Methods.** This retrospective cohort study included all the children born at the Clinic for Gynecology and Obstetrics (Clinical Center of Vojvodina) in Novi Sad during 1996 and 2006. The incidence and the structure of congenital anomalies were analyzed. **Results.** During 1996 there were 6,099 births and major congenital anomalies were found in 215 infants, representing 3.5%. In 2006 there were 6,628 births and major congenital anomalies were noted in 201 newborns, which is 3%. During 1996 there were more children with anomalies of musculoskeletal system, urogenital tract, with anomalies of the central nervous system and chromosomal abnormalities. During the year 2006 there

were more children with cardiovascular anomalies, followed by urogenital anomalies, with significant decline in musculoskeletal anomalies. The distribution of the newborns with major congenital anomalies, regarding perinatal outcome, showed the difference between the studied years. In 2006 the increasing number of children required further investigation and treatment. **Conclusions.** There is no national registry of congenital anomalies in Serbia so the aim of this study was to enlighten this topic. In the span of ten years, covering the period of the NATO campaign in Novi Sad and Serbia, the frequency of major congenital anomalies in the newborns was not increased. The most frequent anomalies observed during both years implied the musculoskeletal, cardiovascular, urogenital and central nervous system. In the year 2006 there was a significant eruption of cardiovascular anomalies and a significant decrease of musculoskeletal anomalies, chromosomal abnormalities and central nervous system anomalies, while the number of urogenital anomalies declined compared to the year 1996.

**Key words:** infant, newborn; congenital abnormalities; epidemiology; risk factors; serbia.

### Apstrakt

**Uvod/Cilj.** Prema Svetskoj zdravstvenoj organizaciji (SZO) kongenitalne anomalije su poremećaji organa ili tkiva, bilo da su vidljive na rođenju ili se manifestuju tokom života, i registrovane su u Međunarodnoj klasifikaciji bolesti. Cilj ove studije bio je da se uporede učestalost i struktura prenatalno utvrđenih i klinički ispoljenih kongenitalnih anomalija novorođenčadi na širem području Novog Sada (Vojvodina, Srbija) u dve udaljene godine (1996. i 2006). **Metode.** Ova retrospektivna studija obuhvatila je svu decu rođenu na Klinici za

ginekologiju i akušerstvo u Novom Sadu (Klinički centar Vojvodine) tokom 1996. i 2006. Analizirani su učestalost i struktura kongenitalnih anomalija. **Rezultati.** Tokom 1996. bilo je 6 099 porođaja, a kongenitalne anomalije registrovane su kod 215 novorođenčadi što predstavlja 3,5% od ukupnog broja novorođenčadi. U 2006. bilo je 6 628 porođaja, a kongenitalne anomalije zabeležene su kod 201 novorođenčeta, što je 3% od ukupnog broja novorođenčadi. Tokom 1996. bilo je više dece sa anomalijama muskuloskeletnog sistema, urogenitalnog trakta, sa anomalijama centralnog nervnog sistema i sa hromozomskim abnormalnostima. Tokom 2006. godine, bilo

je više dece sa anomalijama kardiovaskularnog sistema, slede anomalije urogenitalnog sistema, dok se broj anomalija muskuloskeletnog sistema značajno smanjio. Distribucija novorođenčadi sa velikim anomalijama, u odnosu na perinatalni ishod, pokazala je razliku između ispitivanih godina. U 2006. zabeležen je veći broj dece kod kojih je bila potrebna dalja dijagnostika i lečenje. **Zaključak.** U Srbiji ne postoji nacionalni registar kongenitalnih anomalija i ova studija imala je za cilj da doprinese delimičnom sagledavanju ovog problema. U razmaku od deset godina koji pokriva i period NATO bombardovanja u Novom Sadu i Srbiji, učestalost značajnih kongenitalnih anomalija kod novorođenčadi nije povećana.

Najčešći poremećaji uočeni tokom obe godine bile su malformacije lokomotornog, kardiovaskularnog, urogenitalnog i centralnog nervnog sistema. U 2006. godini došlo je do značajnog porasta broja kardiovaskularnih anomalija i značajnog sniženja broja anomalija lokomotornog sistema, hromozomskih abnormalnosti i anomalija centralnog nervnog sistema, dok je broj urogenitalnih anomalija bio niži u odnosu na 1996.

**Ključne reči:** novorođenčće; anomalije; epidemiologija; faktori rizika; srbija.

## Introduction

According to the World Health Organization (WHO) definition, congenital anomalies are all disorders of the organs or tissues, regardless of whether they are visible at birth or manifest in life, and are registered in the International Classification of Diseases. They can be morphological, structural or functional, hereditary or nonhereditary anomalies, as a result of teratogenic effects of external factors – drugs, infections or toxins in genetically healthy fetus<sup>1-3</sup>. In the occurrence of congenital anomalies, genetic disorders play a role in 20–25%, fetal infection in 3–5%, maternal disease in 2–4%, drugs in pregnancy in < 1%, while the unknown etiological factors reach even 65–75%<sup>4-6</sup>.

Congenital anomalies are divided into major and minor. Major malformations are detected in 2-3% of the newborns before and immediately after birth, and express the same percentage up to fifth year of life (a total of 4–6%). One minor malformation was recorded in 15% of infants and the incidence of major malformations in this group was not higher than in infants without minor malformations<sup>7,8</sup>.

Successful control of acute infectious diseases and nutritional disorders (which are the leading causes of neonatal morbidity and mortality in developing countries) resulted in congenital anomalies becoming the most important cause of perinatal morbidity and mortality in developed countries, where the appropriate level of the epidemiological transition was achieved, *ie*, where the mortality rate in infants was decreased to below 15 deaths *per* 1,000 live births. Such was the case in Vojvodina (northern province of Serbia) where the infant mortality rate is 10.6%. Further decline in infant mortality rates cannot be achieved by existing measures in primary health care of children (immunizations, rational antimicrobial therapy)<sup>9,10</sup>, but with developing of centers for prenatal diagnosis (expert ultrasound, early amniocentesis, cordocentesis, karyotype, biochemical analysis of amniotic fluid)<sup>11,12</sup>.

The aim of the study was to compare the number and structure of clinically manifested congenital anomalies of the newborns in Vojvodina, detected in prenatal period (by the methods mentioned above) or revealed upon physical examination and further follow-up in the newborn period, in the two distant years (1996 and 2006). The study did not cover data concerning abortions due to fetal anomalies.

## Methods

Vojvodina has a total population of about 2 million inhabitants and about 18,000 newborn babies a year. In this paper, the data of the Clinic for Gynecology and Obstetrics in Novi Sad with about 6,500 deliveries annually (Clinical Center of Vojvodina) were used. The study was approved by the Ethic Board and two separate years were analyzed – 1996 and 2006, considering that in 1999 there was the NATO campaign in Serbia, including Novi Sad and the surroundings.

The basic criterion for inclusion in this study was the existence of complete medical records on the number and structure of anomalies, newborn gender, anthropometric parameters, gestational maturity, Apgar score, congenital anomalies, fetal presentation, mode of delivery, parity, perinatal outcome, stillbirth and postnatal mortality.

The prenatal diagnostics was not done routinely (in the screening form) and was indicated only in pregnancies with high risk for congenital anomalies during 1996, while in 2006 it was more precise and complete. Also, the postnatal ultrasound diagnostics of newborns was not yet introduced at the Gynecology and Obstetrics Clinic in Novi Sad in 1996, while in 2006 it was performed upon clinical indications.

The results are presented in tables, and statistical significance was determined using the  $\chi^2$  test, *t*-test and Fisher test.

## Results

At the Clinic for Gynecology and Obstetrics in Novi Sad (Clinical Center of Vojvodina) during 1996 there were 6,099 births and major congenital anomalies were found in 215 infants, representing 3.5%. In 2006, there were 6,628 births and major congenital anomalies were noted in 201 newborns, what was 3% of the total number of newborns. The gender structure of newborns with major congenital anomalies in 1996 and 2006 was about the same (Fisher test,  $p > 0.05$ ). Comparing the mean values of body mass and length, newborns with major congenital anomalies in 1996 had a greater average body weight ( $p < 0.01$ ) and length ( $p < 0.01$ ). There was no difference in the average circumference of the head ( $p > 0.05$ ) between the two groups of babies. Apgar scores in the first ( $p < 0.01$ ) and the fifth minute ( $p < 0.01$ ) significantly differed between the two groups. Children born in 1996 had higher average values of Apgar scores in the first and fifth minute of life. Anthropometric

parameters and final outcome among newborns with congenital anomalies are shown in Table 1. It is visible that in 2006 more newborns were transferred to the Institute for Children and Youth Health Care for further investigation (37.3% which was twice as more as in 1996) and followed-up till the end of the newborn period.

Analysis of the structure of major congenital anomalies showed that the total incidence of major congenital anomalies was almost the same, but there was a significant difference in its distribution.

The most frequent abnormalities observed during both years implied the musculoskeletal, cardiovascular, urogenital and central nervous system. In the year 2006 there was a significant eruption of cardiovascular anomalies ( $p < 0.01$ ) and a significant decrease of musculoskeletal anomalies, chromosomal and

central nervous system anomalies. The number of urogenital anomalies in both years was at the second position (in 1996 involved 35.8% and declined in 2006 to 24.8%). During 1996 there were more children with anomalies of musculoskeletal system ( $p < 0.01$ ), with anomalies of the central nervous system ( $p < 0.01$ ) and chromosomal abnormalities ( $p < 0.01$ ).

The detailed structure of major anomalies in the newborn infants in Novi Sad in 1996 and 2006 is shown in Table 2.

Most of the infants (70.4% in 1996 and 67.6% in 2006) with major anomalies diagnosed at birth, belonged to the normal zone of gestational age and body mass (*temporarius/eutrophicus*), with no differences observed between the two years (Fisher test,  $p > 0.05$ ) (Table 3).

Among the group of the newborns with major congenital anomalies, 25 (11.6%) born in 1996 and 22 (10.9%) born

**Table 1**  
Characteristics of the infants with major congenital anomalies in Novi Sad in 1996 and 2006

Infants characteristics	1996	2006
Total number of children, n (%)	215	201
males	96 (44.6)	108 (53.7)
females	119 (55.4)	93 (46.2)
Birth weight (g)*	3376.43	3065.08
Birth length (cm)*	49.60	48.70
Head circumference (cm)*	33.8	33.48
Apgar score 1 min.*	8.98	9.57
Apgar score 5 min.*	8.12	9.17
Perinatal outcome, n (%)		
discharged	181 (84.2)	126 (62.7)
transferred	34 (15.8)	75 (37.3)

\*mean values.

**Table 2**  
Structure of major anomalies in the newborn infants in Novi Sad in 1996 and 2006

Major anomalies	1996		2006	
	n	%	n	%
Cardiovascular system	56	26.0	110	54.7
Urogenital system	77	35.8	50	24.8
Eye	0	0.0	1	0.5
Abdominal wall/organs	6	2.8	10	5.0
Central nervous system	25	11.6	7	3.5
Musculoskeletal system	112	52.1	44	21.9
Chromosomopathia	14	6.5	7	3.5
Face	2	0.9	2	1.0
Respiratory system	0	0.0	1	0.5
Multiple (two or more) major anomalies	29	0.13	31	0.15

**Table 3**  
Categorization of newborns with major congenital anomalies according to gestational age and body mass (growth) in 1996 and 2006

Body mass (growth)	<i>Pretemporarius</i> , n (%)		<i>Temporarius</i> , n (%)		<i>Posttemporarius</i> , n (%)	
	1996	2006	1996	2006	1996	2006
<i>Hypotrophicus</i>	1 (0.5)	6 (3.0)	17 (7.9)	7 (3.4)	0 (0.0)	0 (0.0)
<i>Eutrophicus</i>						
< 5 p	0 (0.0)	3 (1.5)	7 (3.2)	6 (3.0)	0 (0.0)	0 (0.0)
< 10 p	13 (6.0)	25 (12.4)	151 (70.4)	136 (67.6)	1 (0.5)	0 (0.0)
<i>Hypertrophicus</i>						
> 90 p	0 (0.0)	0 (0.0)	15 (6.9)	8 (4.0)	0 (0.0)	0 (0.0)
> 95 p	2 (0.9)	0 (0.0)	8 (3.7)	10 (4.9)	0 (0.0)	0 (0.0)
Total	16 (7.4)	34 (16.9)	198 (92.1)	167 (82.9)	1 (0.5)	0 (0.0)

p – growth percentile.

in 2006 had intrauterine growth restriction (IUGR). Hypertrophic intrauterine growth had 25 (11.5%) of the neonates born in 1996 and 18 (8.9%) born in 2006. In 1996, hypertrophic intrauterine growth was present in 17 newborns with anomalies of musculoskeletal system, then in 6 newborns with anomalies of the cardiovascular system and in 1 newborn with anomaly of the anterior abdominal wall and abdominal organs and 1 with anomalies of the urogenital system. In 2006, hypertrophic intrauterine growth was noticed in 10 of the newborns with cardiovascular anomalies, in 4 of the newborns with anomalies of the musculoskeletal system, in 3 of the newborns with anomalies of the anterior abdominal wall and abdominal organs, and in 1 infant with anomalies of the urogenital system.

In 2006 an increase in the incidence of breech presentation was observed. Vaginal delivery was applied for 167 (77.67%) children in 1996, and for 118 (58.7%) in 2006. Delivery with intervention (*sectio Caesarea* and vacuum extraction) was performed in 48 (22.33%) births during 1996 and in 83 (41.3%) births in 2006 (Fisher test,  $p < 0.05$ ). The distribution of the newborns with major congenital anomalies regarding perinatal outcome showed a significant difference between the studied years. In 2006, the increasing number of children required further investigation and treatment (Fisher test,  $p < 0.05$ ). Related to the two observed years there were no significant differences between primiparas

and multiparas concerning newborns with congenital abnormalities ( $p > 0.05$ ) (Table 4).

The number of newborns with multiple major anomalies was nearly identical in both years. During 1996 multiple anomalies were recorded in 29 (0.13%), and in 2006 in 31 (0.15%) of the newborns.

The analysis of autopsy records (Institute of Histology and Pathology, Clinical Center of Vojvodina in Novi Sad, Serbia) revealed 150 registered stillbirths and neonatal deaths during 1996 (of which 23 children with congenital anomalies) and 75 children without anomalies, and 52 children that were not taken into analysis due to incomplete data. In 2006 there were 221 stillbirths and neonatal deaths, 54 of them with and 167 without congenital anomalies. Congenital anomalies were more frequently observed among stillborn children in the year 2006 ( $p < 0.01$ ) (Table 5).

There was no significant difference in the gender structure of stillbirths and neonatal deaths between the two observed years ( $p > 0.05$ ). The gestational age of stillborn children between the studied years was not significantly different. Structural analysis of the anomalies of the specific organ systems found in stillbirths and postnatal deaths of children up to 28 days of age in 1996 and 2006 year, showed the highest incidence of abdominal wall anomalies/organs, central nervous system or multiple anomalies (Table 6).

**Table 4**  
**Perinatal characteristics of the newborns with congenital anomalies in Novi Sad in 1996 and 2006**

Perinatal characteristics	1996	2006
	n (%)	n (%)
Presentation		
occipital	205 (95.4)	188 (93.5)
breech	9 (4.1)	13 (6.5)
vertex	1 (0.5)	0 (0.0)
Completion of delivery		
vaginal-occipital presentation	156 (73.0)	116 (58.2)
vaginal-breech presentation	9 (4.2)	2 (1.0)
vaginal-vertex presentation	2 (0.9)	0 (0.0)
sectio cesarea- occipital presentation	41 (18.6)	72 (35.4)
sectio cesarea-breech presentation	0 (0.0)	10 (4.9)
vacuum extraction-occipital presentation	7 (3.3)	1 (0.5)
Partity of mother		
primiparous	104 (48.4)	118 (58.7)
multiparous	111 (51.6)	83 (41.3)

**Table 5**  
**Presence of anomalies in stillbirths and neonatal deaths in Novi Sad in 1996 and 2006**

Presence of anomalies	Stillbirths, n (%)		Neonatal deaths, n (%)		Total, n (%)	
	1996	2006	1996	2006	1996	2006
Anomaly	16 (13.4)	47 (24.6)	7 (22.6)	7 (23.3)	23 (15.3)	54 (24.4)
No anomaly	65 (54.7)	144 (75.4)	10 (32.2)	23 (76.7)	75 (50.0)	167 (75.6)
Complete data lacking	38 (31.9)	0 (0)	14 (45.2)	0 (0)	52 (34.7)	0 (0)
Total	119 (100.0)	191 (100.0)	31 (100.0)	30 (100.0)	150 (100.0)	221 (100.0)

**Table 6**

**Characteristics of stillbirths and neonatal deaths in Novi Sad in 1996 and 2006**

Characteristics of stillbirths and neonatal deaths	1996		2006	
	n	%	n	%
Total number of stillbirths and neonatal deaths	23	100.0	54	100.0
Gender structure				
male	11	47.8	25	46.3
female	12	52.2	27	50.0
ambiguous	0	0.0	2	3.7
Anomalies				
urogenital system	2	8.7	3	6.4
chromosomopathia	0	0.0	8	17.0
central nervous system	5	21.7	6	12.8
face	2	8.7	3	6.4
cardiovascular system	1	4.3	5	10.6
abdominal wall/organs	6	26.1	11	23.4
musculoskeletal system	2	8.7	7	14.8
multiple	5	21.7	11	23.4

## Discussion

There is no national registry of congenital anomalies in Serbia and the aim of this study was to enlighten this topic. The overall prevalence of congenital anomalies in the studied population of newborns in the area of Novi Sad (Vojvodina, Serbia) is congruent with the references showing that the incidence of anomalies in the population is about 5%, of which major anomalies participate with about 3%<sup>7, 13, 14</sup>.

Comparing the two observed years, in 2006 there was a convincing change in the structure of the frequency of specified major anomalies of newborns. Anomalies of the urogenital system in 1996 were represented in 35.8% and in 2006 in 24.8% newborns. According to the WHO, anomalies of the urogenital system are presented with 18% of the overall structure of anomalies. There was a convincing increase in cardiovascular anomalies, from 26% during 1996 to 54.7% in 2006, which is beyond the literature data on their incidence of 25%. A significant decrease in 2006 was recorded when it comes to musculoskeletal anomalies (21.9%), which dominated in 1996 with the percentage distribution of 52.1%, which exceeds the cited numbers of 22%. The decrease was registered and when it comes to chromosomal abnormalities, which in 1996 had the presence in 6.5%, while in 2006, in 3.5% of newborns. In summary, in 2006 there were more children with anomalies of the cardiovascular system, while in 1996 there were more children with anomalies of the musculoskeletal system, urogenital and central nervous system and chromosomal abnormalities. In 2006 it was found the occurrence of a major anomaly of the eye and respiratory systems, which are not registered in 1996, while major malformations of the face, jaw and palate did not exceed the most common literature values. The WHO data indicate that the most frequent anomalies are those of cardiovascular, musculoskeletal, genitourinary and of the central nervous system<sup>9, 15, 16</sup>.

Low birth weight could be expected in newborns with structural anomalies, which in this study, except in one case, was not registered. There were a significantly higher body

birth weight and length of newborns in 1996 than in 2006, but they did not deviate from the average values registered in 90% of newborns (birth weight 2500–4100 g, body length 46–54 cm, head circumference 33–36 cm)<sup>17–19</sup>, except newborns with anomalies of the respiratory and musculoskeletal systems, where these average values were low.

Significant differences in the gender structure of the examined newborns with major congenital anomalies were not recorded between the observed years, nor within individual organ systems, which would indicate the absence of sexual dimorphism, except the locomotor system abnormalities that were two times more frequent in female infants in 1996 and three times more frequent, also in female newborns, in 2006. This corresponds to literature data on their higher prevalence in female children, related primarily to developmental hip dysplasia, which is dominated by the anomalies of musculoskeletal system<sup>18–21</sup>.

Worldwide, the incidence of premature live births varies widely, depending on geographic location, racial origin and development of the country, so in the UK it is 4.6%<sup>10, 20, 21</sup>, 12–13% in the USA, while in other developed countries is 5–9%. Certainly, premature labor is an important factor for fetal damage and it is reasonable for the greater number of congenital anomalies in premature than in term newborns. Increased risk of preterm delivery was found in congenital anomalies such as anencephaly, renal agenesis, atresia of the small intestine, omfalocela, gastroschisis, tracheoesophageal fistula, oesophageal atresia etc<sup>11, 22, 23</sup>.

Intrauterine growth restriction is one of the most common disorders in pregnancy, which significantly increases the risk of intrauterine mortality and morbidity in newborns. The frequency of IUGR in Europe ranges from 4.1% in Finland to 9.5% in Romania, while in developing countries it exceeds 10%, and the values obtained in this study are close to those in Europe. Twin pregnancies account for about 20% of cases of IUGR, chromosomal abnormalities were detected in approximately 15% of cases of IUGR, uteroplacental insufficiency, fetal infection and fetal exposure to radiation together constitute about 10% of IUGR, while the remaining

55% of cases are bone dysplasias (achondroplasia), osteogenesis imperfecta, immune and other reasons<sup>12, 24, 25</sup>.

Hypertrophic intrauterine growth was seen in 10% of births. In hypertrophic newborns the occurrence of hip subluxation and talus calcaneovalgus was more frequent. Hypertrophic growth is common with hydrocephalus and the syndromes with hyperinsulinism (Beckwith-Wiedemann syndrome, Sotos syndrome, infants of diabetic mothers)<sup>12, 13, 26</sup>. In 1996 hypertrophic intrauterine growth was more frequent and associated with anomalies of the locomotor system, than the cardiovascular system and anomalies of the anterior abdominal wall, abdominal organs, and urogenital system. In 2006, hypertrophic intrauterine growth was mostly present in newborns with anomalies of the cardiovascular system, musculoskeletal system, anterior abdominal wall, abdominal organs and the urogenital system.

Apgar scores (both the first and fifth minute) were higher in 1996, showing the more severe clinical pattern of anomalies in 2006. In both observed years, Apgar score was higher in the fifth minute of life, indicating a good adaptability of infants with major congenital anomalies to the extrauterine life.

The association of multiple organ system anomalies indicates the effect of teratogenic agents during the first trimester of pregnancy. A small number of live born infants with associated major anomalies was found in this study, as in others with the prevalence of 0.7%<sup>18, 27</sup>.

The incidence of breech presentation in the world is 3–4%, and in the newborns with congenital anomalies it is often present because the malformed fetus is not able to take a stand head and pelvic posture may be an indicator for the presence of anomaly<sup>14, 28</sup>. Breech presentation was more common in newborns with musculoskeletal, cardiovascular and chromosomal abnormalities, in accordance with the reference data<sup>15, 29, 30</sup>.

The fact that more deliveries with cardiovascular and urogenital congenital anomalies in newborns in 2006 were completed with intervention (compared to 1996), speaks in favor of the better prenatal diagnosis of these defects. Caesarean section, as an intervention during childbirth, except for the usual obstetric indications, should be used with breech fetal presentation in primipara or in imminent preterm deliveries, regardless of parity. It is, however, believed that abo-

ut 40% of breech deliveries can be performed vaginally without compromising neonatal outcomes<sup>17</sup>.

In relation to parity, no differences in the frequency of anomalies were found which is consistent with the reference data.

Comparing to 1996, in 2006 the increased number of the newborns was transferred from the Department of Gynecology and Obstetrics to the Institute for Children and Youth Health Care in Novi Sad, on further examination and treatment, indicating the greater severity of anomalies. The largest number of the newborns with structural defects was in the age group of mothers between 18 and 35 years, which requires changes in attitudes in prenatal screening. Introduction of amniocentesis in women older than 35 years has led to a significant reduction in the birth of children with chromosomal abnormalities in this age group, but it still remained a problem among young pregnant women who were not covered by mandatory screening<sup>31</sup>.

A significantly higher number of stillbirths and neonatal deaths in 2006 compared to 1996 year speaks for the severity of anomalies.

### Conclusion

In the span of ten years, covering the period of the NATO campaign in Novi Sad and the environment, the frequency of major congenital anomalies in the newborns was not increased.

The most frequent anomalies observed during both years implied the musculoskeletal, cardiovascular, urogenital and central nervous system. In the year 2006 there was a significant eruption of cardiovascular anomalies and a significant decrease of musculoskeletal anomalies, chromosomal and central nervous system abnormalities, while the number of urogenital anomalies declined from one third in 1996 to one fourth in 2006.

In the year 2006 there was an obvious increase in breech presentation in the newborns with major congenital anomalies, compared to the year 1996. Higher Apgar score in the fifth minute of life in the year 2006 indicated a good adaptability of infants with major congenital anomalies, but also a higher level of perinatal care.

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## Association of vascular endothelial growth factor expression with pathohistological parameters of cutaneous melanoma

Udruženost ekspresije vaskularnog endotelnog faktora rasta sa patohistološkim parametrima kožnih melanoma

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### Abstract

**Background/Aim.** Melanoma is the most aggressive malignant tumor of the skin. Contradictory data was published on vascular endothelial growth factor (VEGF) in tumor samples and its role in skin melanoma progression and prognosis. The aim of this study was to investigate the significance of VEGF expression as a prognostic parameter in melanoma. **Methods.** The experimental group included 81 patients with primary skin melanomas treated from 2009 to 2013 at the Military Medical Academy, Belgrade. The control group included 20 patients with dysplastic and 20 with benign naevi. Stratification was done according to gender, age, clinical and pathological stage, localization, histologic type, Clark's, Breslow, mitotic count, regression and ulceration, tumor infiltrating lymphocytes and metastatic spread. Immunohistochemical staining was performed on skin biopsies using DAKO anti-VEGF antibodies (Ab), LSAB<sup>TM</sup> +HRP, DAB and microwave antigen (Ag) retrieval in DAKO pH 9.0 solution. For statistical data analysis was done with ANOVA, Bonferroni, Mann Whitney and Wilcoxon test. **Results.** The mean intensity of VEGF staining was statistically significantly higher in melanomas than in

benign or dysplastic naevi. Furthermore, the highest recorded values were in Ia and IV clinical stages. The majority of melanomas with high intensity of VEGF staining were in pT1a pathological stage. Melanomas with the highest mitotic count (> 6) had a significantly higher intensity of VEGF staining than those with < 2 mitoses. The highest intensity of staining was in melanomas without significant lymphocytic infiltrate and the lowest was in those with brisk lymphocytic infiltrate, thus a statistical difference was significant. The mean intensity of VEGF staining was highest in melanomas with lymphovascular invasion. There was no statistically significant difference between VEGF and any other parameter. **Conclusion.** VEGF in primary skin melanomas plays an important role in tumor progression and is linked to the absence of tumor infiltrating lymphocytes and the presence of lymphovascular invasion. More detailed studies have to be done on VEGF prognostic value in melanoma on a larger number of patients.

**Key words:** melanoma; skin; vascular endothelial growth factors; histology; immunohistochemistry; sensitivity and specificity.

### Apstrakt

**Uvod/Cilj.** Melanom je najagresivniji maligni tumor kože. Do sada su objavljeni kontradiktorni podaci o vaskularnom endotelnom faktoru rasta (VEGF) prisutnom u uzorcima tumora, kao i njegovoj ulozi u progresiji i prognozi melanoma kože. Cilj ove studije bio je da se ispita značaj ekspresije VEGF kao prognostičkog parametra kod melanoma. **Metode.** Eksperimentalna grupa sastojala se od 81 bolesnika sa primarnim melanomom kože lečenih na Vojnomedi-

cinskoj akademiji, Beograd, od 2009. do 2013. godine. Kontrolna grupa sastojala se od 20 bolesnika sa displastičnim i 20 sa benignim nevusima. Stratifikacija je izvršena prema polu, starosti, kliničkom i histološkom stadijumu, lokalizaciji, histološkom tipu, Clark-u, Breslovu, broju mitozu, regresiji i ulceraciji, prisustvu tumorinfiltrirajućih limfocita i načinu širenja. Imunohistohemijsko bojenje izvedeno je na biopsijama kože uz upotrebu DAKO anti-VEGF antitela, LSAB<sup>TM</sup> + HRP, Liquid DAB i mikrotalasnog demaskiranja u DAKO pH 9.0 rastvoru. Za statističku obradu korišćeni



su testovi ANOVA, Bonferroni post-test, Mann-Whitney i Wilcoxon test. **Rezultati.** Prosečan intenzitet VEGF bojenja bio je značajno veći u melanomima nego u benignim ili displastičnim nevusima, a u melanomima bio je najveći u Ia i IV kliničkom stadijumu. Većina melanoma u pT1a patološkom stadijumu imala je najveće prosečne vrednosti VEGF. Melanomi sa najvećim brojem mitoze (> 6) imali su značajno veći intenzitet VEGF bojenja nego oni sa < 2 mitoze. Melanomi bez limfocitne infiltracije imali su najveće vrednosti VEGF, dok su oni sa najintenzivnijom infiltracijom imali najniže vrednosti. Ova razlika je bila statistički visokoznačajna. Melanomi sa limfnim i hematogenim načinom širenja imali su najveće prosečne vrednosti VEGF. Nije bilo

statistički značajne korelacije između intenziteta VEGF bojenja i bilo kog drugog parametra. **Zaključak.** U primarnim melanomima kože povećana ekspresija VEGF značajna je za tumorsku progresiju, a povezana je kako sa odsustvom limfocitne infiltracije, tako i sa prisustvom limfovaskularne invazije. Potrebna su detaljnija prospektivna istraživanja ekspresije VEGF kao prognostičkog parametra kod melanoma na većem broju bolesnika.

**Ključne reči:**  
**melanom; koža; vaskularni endotelni faktori rasta; histologija; imunohistohemija; osetljivost i specifičnost.**

## Introduction

Melanoma is the most aggressive malignant tumor of the skin. In the period from 1999 to 2008 the Cancer Registry of Central Serbia reported the incidence of melanoma to be 1.4% for men and 1.5% for women. According to the same source, melanoma was recoded as the main cause of death in 1.1% of the population<sup>1</sup>. Patients with stage III and IV disease had a 5-year survival rate of only 14% due to exceptionally high chemo- and radiotherapy resistance of melanomas<sup>2</sup>. Surgical resection of primary tumor at an early stage (*in situ* melanomas) is usually curable, but treatment for metastatic melanoma is still a challenge. Numerous genetic and biological studies of factors relevant for pathogenesis and progression of melanoma may be split into two groups. The first group relies on the identification of new reliable biomarkers of early melanoma progression, predictive and prognostic factors, while the second is dependent on the identification of resources and/or procedures to reduce chemoresistance, to induce apoptosis and decrease proliferation and metastatic potential of melanoma cells<sup>3-5</sup>. Angiogenesis plays a significant role in the progression and metastasis of melanoma<sup>6-8</sup>. Tumor angiogenesis is a net outcome of an interplay between pro-angiogenic and inhibiting factors of angiogenesis<sup>8</sup>. A key event in the activation of angiogenesis is an increase in angiogenic factors and/or decrease/loss of inhibitors of angiogenesis. Hypoxia is one of the physiological stimuli responsible for the increase in production of various pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF). Expression of VEGF is triggered by hypoxia-induced factor-1 $\alpha$  (HIF1 $\alpha$ ), the oxygen-sensitive transcription factor also known as angiogenic trigger (angiogenic switch). HIF1 $\alpha$  is activated by low pO<sub>2</sub>, low pH, hyper/hypoglycemia, hyperthermia, mechanical stress, immune/inflammatory response or by genetic mutations<sup>9,10</sup>. The activity of pro- and anti-angiogenic factors is regulated by multiple genes, many of these often mutated in cancers. For example, in normal cells, p53 can stimulate the expression of anti-angiogenic molecules, such as thrombospondin-1, and inhibit the expression of pro-angiogenic molecules such as VEGF. Thus, the loss of p53 in tumor cells not only affects the cell cycle, but also angiogenesis. Transcription of VEGF is also regulated by the RAS-MAP kinases signals and mutations in RAS and MYC genes.

The role of VEGF in metastatic melanoma has been the main focus of research in recent years<sup>11-15</sup>. It has been shown that VEGF stimulates tumor angiogenesis in autocrine and paracrine fashion. VEGF is secreted by melanoma cells, stromal cells, dendritic cells, macrophages, and fibroblasts. A number of members of the VEGF family are identified: VEGF-A, B, C, D, E, and placental growth factor (placenta growth factor – PlGF). Different forms of VEGF bind to the VEGF-receptor with tyrosine kinase activity<sup>6</sup>. VEGF stimulates endothelial cell proliferation, migration, vasodilatation and vasculogenesis and recruitment of hematopoietic progenitor cells in the bone marrow<sup>16,17</sup>.

Contradictory data was published on VEGF in tumor tissue samples and its role in melanoma progression and prognosis<sup>18,19</sup>. In previous publications VEGF was analyzed in melanoma tissue or in blood serum of melanoma patients, but a very small number of studies was done simultaneously on both tumor tissue and serum obtained from the same patient. The therapy based on inhibition of the VEGF signaling pathway so far appears promising, although further research is needed. Main prognostic factors are melanoma thickness (Breslow score), ulceration, sentinel lymph node status and anatomical site, the latter considered to be an independent prognostic factor in primary melanoma<sup>15</sup>. The aim of this study was to introduce VEGF-A as a reliable new prognostic factor in melanoma, to compare this new factor with those already in use and to examine the link between initial expression of VEGF with local tumour progression and metastatic spread.

Penetration of blood or lymph vessels depends on the profile of generated chemotactic and angiogenic factors and physical tissue barriers<sup>20</sup>. Blood and lymphatic vessels share a common embryologic origin, and they respond to the same growth factors: VEGF-A, VEGF-C, VEGF-D, fibroblast growth factor (FGF2), platelet-derived growth factor (PDGF-A), hepatocyte growth factor (HGF), etc.<sup>21</sup>. It is, therefore, to be expected that tumors simultaneously induce lymphangiogenesis and angiogenesis<sup>22</sup> but, for an unknown reason, this is often not the case. Proliferation of lymphatic vessels was detected in melanoma<sup>22</sup>, as well as in squamous cell carcinomas of the head and neck<sup>23</sup> while in other cancers it is poorly documented. A possible reason for the lack of proliferation of lymphatics in tumours may be caused by

anti-lymphangiogenic factors<sup>24</sup>. Vascular endothelial growth factor C (VEGF-C) released by macrophages and melanoma cells adjacent to the main tumor mass is the key lymphangiogenic factor. Skin metastases, subcutaneous and lymph node metastases were associated with longer survival than visceral metastases. In recent years it has been established that chemokines and their receptors play a major role in metastasis<sup>25,26</sup> and that certain chemokines and their receptors are directly involved in molecular control mechanisms in the spread of melanoma<sup>27</sup>.

### Methods

Tissue samples of cutaneous melanoma were obtained from 81 patients aged between 23 and 87, diagnosed between 2009 and 2013 at the Institute of Pathology and Forensic Medicine of Military Medical Academy in Belgrade, Serbia. The distribution of patients according to clinical stage of melanoma was 22 in stage I, 18 in stage II, 27 in stage III and 13 in stage IV. The control group comprised of 20 patients with dysplastic naevi and 20 patients with benign melanocytic naevi.

Out of 81 patients, 43 were males and 38 females. All the patients were stratified by age, sex, clinical stage, histological TNM stage, Clark's level, Breslow score, histologic type of melanoma, mitotic count, the presence of tumor infiltrating lymphocytes (TIL), anatomical localization and route of spread (lymphatic, hematogenous or combined).

Immunohistochemical staining with mouse monoclonal anti-human VEGF antibody (Ab) (DAKO clone VG1, dilution 1 : 25) was performed on surgical skin biopsies that were fixed in 10% neutral buffered formalin and embedded in pa-

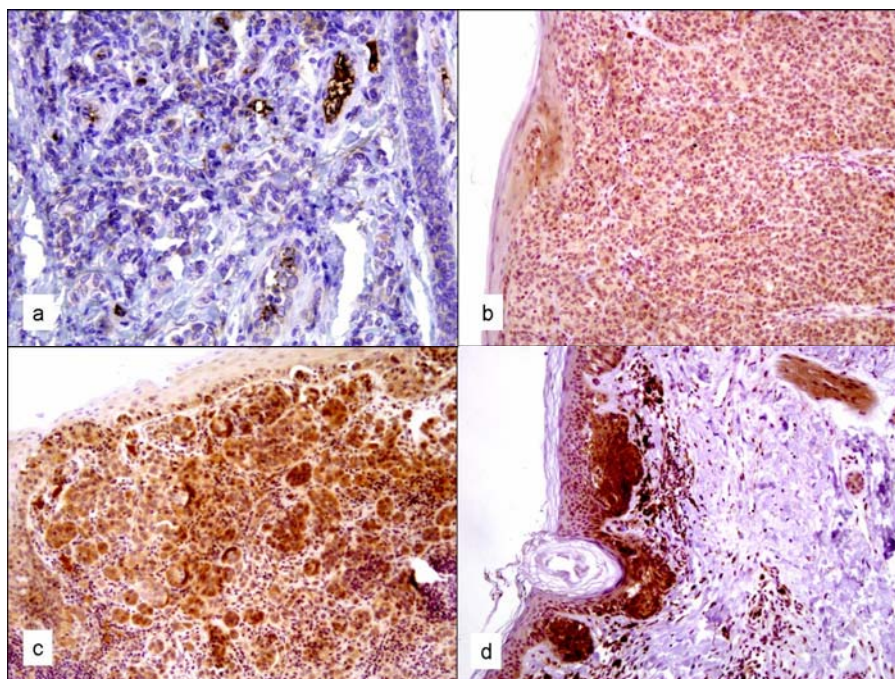
raffin. Antigen (Ag) retrieval was performed using a pH 9.0, DAKO Code S2367 Ag retrieval solution and microwave oven. We used a LSAB<sup>TM</sup> + labelling system HRP and DAKO Liquid DAB + substrate chromogen system. For negative controls primary Ab was replaced by mouse IgG1 DAKO Ab, diluted to the same concentration as the primary antibody. Keratinocytes in the epidermis adjacent to melanomas served as VEGF internal positive control. The intensity of immunohistochemical staining was scored semiquantitatively from 0–3 according to the publication of Rajabi et al.<sup>19</sup>: 0 – no difference in staining between malignant melanocytes and keratinocytes; 1 – staining slightly more intense than in keratinocytes; 2 – staining moderately more intense than in keratinocytes, and 3 – staining more intense than in keratinocytes (Figure 1).

Statistical analysis of our data was performed with GraphPad Prism software using ANOVA test (with Bonferroni post testing), Mann Whitney (MW) test and Wilcoxon test.

### Results

VEGF expression in correlation with clinical stage showed that 89% of melanomas in the earliest clinical stage had the strongest intensity of staining for VEGF (Table 1), even stronger than melanomas in stage IV (84%) (Table 1). There was no VEGF expression in 15% of primary melanomas in stage Ib and 40% of melanomas in stage IIa (Table 1). As expected, we found that all primary melanomas with metastatic disease (clinical stages III and IV) were positive for VEGF, although the intensity of this staining was variable.

VEGF values in tumor stage IA were significantly higher



**Fig. 1 – Vascular endothelial growth factor (VEGF) in primary melanomas: a) score 0, magnification 200 ×; b) score 1, magnification 100 ×; c) score 2, magnification 100 ×; d) score 3, magnification 100×.**

than in the tumor samples in stages IIA, IIB, IIC and IIIA ( $p = 0.0175$ , MW test) (Figure 2a). In addition, VEGF in tissue samples of patients with stage IV was significantly higher than in samples of patients in stages IB, IIA, IIB, IIC, IIIA and IIIB.

Analysis of VEGF expression according to the histological staging showed similar results, whereby the majority of patients

with strongest intensity of VEGF staining belonged to the group with the earliest pT1a histological stage (Table 1). The average levels of VEGF expression were high in the patients with pT1a, pT2a, pT3b and pT4b stages, with the highest absolute value in pT3b group (Table 2). The average degree of VEGF expression in pT2a stage was significantly higher than in pT2b ( $p = 0.0346$ ,

**Table 1**  
**Frequency of vascular endothelial growth factor (VEGF) expression in melanoma samples**

Variables	VEGF immunostaining level, n (%)			
	0	1	2	3
<b>Clinical stage</b>				
Ia	0 (0)	1 (11)	0 (0)	8 (89)
Ib	2 (15)	1 (8)	5 (38)	5 (38)
IIa	2 (40)	1 (20)	1 (20)	1 (20)
IIb	0 (0)	2 (40)	2 (40)	1 (20)
IIc	1 (12)	3 (38)	2 (25)	2 (25)
IIIa	0 (0)	9 (39)	6 (26)	8 (35)
IIIb	0 (0)	1 (25)	2 (50)	1 (25)
IV	0 (0)	1 (8)	1 (8)	11 (84)
<b>Histological stage</b>				
pT1a	0 (0)	1 (13)	0 (0)	7 (87)
pT1b	2 (66)	0 (0)	1 (33)	0 (0)
pT2a	0 (0)	0 (0)	4 (36)	7 (64)
pT2b	0 (0)	1 (33)	1 (33)	1 (33)
pT3a	1 (25)	1 (25)	1 (25)	1 (25)
pT3b	0 (0)	2 (66)	1 (33)	0 (0)
pT4a	1 (13)	2 (29)	2 (29)	2 (29)
pT4b	0 (0)	8 (29)	6 (21)	14 (50)
<b>Mitoses</b>				
0	1 (3)	8 (22)	9 (24)	19 (51)
1	4 (24)	5 (28)	4 (24)	4 (24)
2	0 (0)	1 (11)	3 (33)	5 (56)
3	0 (0)	1 (25)	0 (0)	3 (75)
4	0 (0)	2 (50)	0 (0)	2 (50)
6	0 (0)	1 (20)	2 (40)	2 (40)
> 6	0 (0)	0 (0)	1 (25)	3 (75)
<b>Lymphocyte infiltration</b>				
0	0 (0)	6 (23)	6 (23)	14 (54)
1	3 (14)	4 (19)	6 (29)	8 (38)
2	2 (7)	8 (30)	5 (19)	12 (44)
3	2 (33)	2 (33)	1 (17)	1 (17)
<b>Clark</b>				
I	/ /	/ /	/ /	/ /
II	2 (20)	1 (10)	1 (10)	6 (60)
III	0 (0)	4 (17)	6 (26)	13 (57)
IV	2 (8)	4 (15)	9 (35)	11 (42)
V	1 (11)	4 (44)	2 (22)	2 (22)
<b>Spreading</b>				
no	5 (11)	9 (21)	8 (19)	21 (49)
lymphatic	0 (0)	7 (29)	9 (38)	8 (33)
lympho-hematogenous	0 (0)	0 (0)	2 (22)	7 (78)
<b>Anatomical localisation</b>				
head	0 (0)	0 (0)	3 (25)	9 (75)
trunk	0 (0)	11 (30)	10 (28)	15 (42)
arm	4 (100)	0 (0)	0 (0)	0 (0)
leg	1 (6)	5 (28)	4 (22)	8 (44)
foot	0 (0)	0 (0)	2 (50)	2 (50)

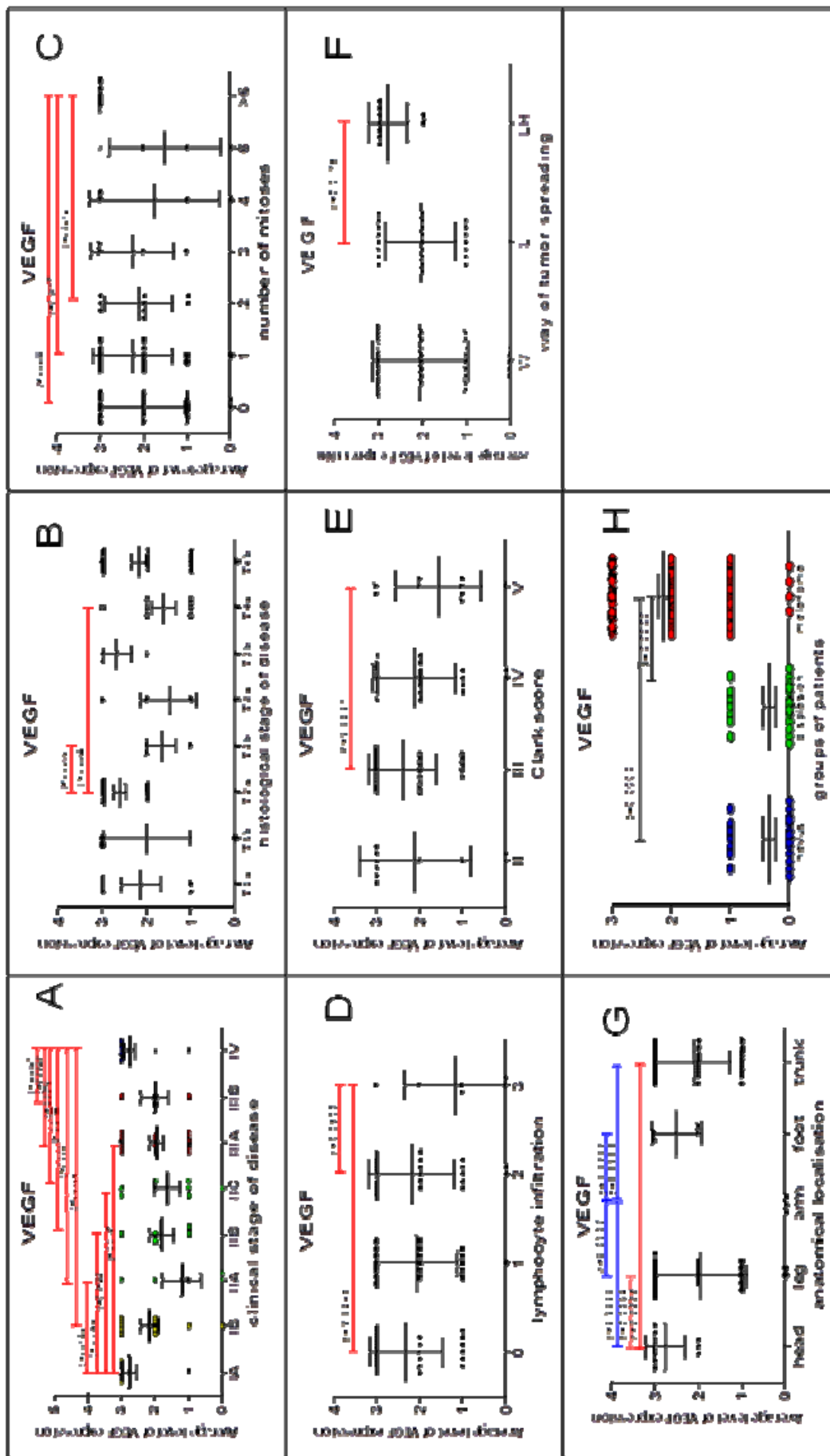


Fig. 2 – Average level of tumor vascular endothelial growth factor (VEGF) expression in the investigated groups: A) Average level of VEGF expression vs clinical stage of disease; B) Average level of VEGF expression vs histological stage of disease; C) Average level of VEGF expression vs number of mitoses; D) Average level of VEGF expression vs lymphocyte infiltration; E) Average level of VEGF expression vs Clark score; F) Average level of VEGF expression vs way of tumor spreading; G) Average level of VEGF expression vs anatomical localisation; H) Average level of VEGF expression vs groups of patients.

W – without spreading; L – lymphatic way of spreading; LH – lymphatic and hematogenous way of spreading.

**Table 2**  
Average level of vascular endothelial growth factor (VEGF) tissue expression in primary melanomas and control groups according to the investigated parameters

Clin stage,	IA	IB	IIA	IIB	IIC	IIIA	IIIB
$\bar{x} \pm SD$	2.78 ± 0.67	2.17 ± 0.94	1.20 ± 1.30	1.80 ± 0.84	1.63 ± 1.06	1.96 ± 0.88	2.00 ± 0.82
significance	IA / IIA $p = 0.0159$	IB / IV $p = 0.409$	IIA / IV $p = 0.0081$	IIB / IV $p = 0.0155$	IIC / IV $p = 0.0076$	IIIA / IV $p = 0.0062$	IIIB / IV $p = 0.0407$
	IA / IIB $p = 0.0286$						
	IA / IIC $p = 0.0169$						
	IA / IIIA $p = 0.0167$						
Hist stage,	pT1a	pT1b	pT2a	pT2b	pT3a	pT3b	pT4a
$\bar{x} \pm SD$	2.13 ± 1.25	2.00 ± 1.73	2.63 ± 0.51	1.67 ± 0.58	1.50 ± 1.29	2.67 ± 0.58	1.63 ± 0.74
significance			pT2a / pT4a $p = 0.0065$				
			pT2a / pT2b $p = 0.0346$				
Mitoses,	0	1	2	3	4	6	> 6
$\bar{x} \pm SD$	2.24 ± 0.96	2.38 ± 0.96	2.44 ± 0.73	2.00 ± 1.15	2.00 ± 1.41	2.00 ± 1.42	2.75 ± 0.50
significance	0 / > 6 $p = 0.0200$	1 / > 6 $p = 0.0417$	2 / > 6 $p = 0.0319$				
Ly Infiltr,	0	1	2	3			
$\bar{x} \pm SD$	2.31 ± 0.84	2.05 ± 0.95	2.19 ± 1.00	1.17 ± 1.17			
significance	0 / 3 $p = 0.0245$		2 / 3 $p = 0.0500$				
Clark,	I	II	III	IV	V		
$\bar{x} \pm SD$	none	2.10 ± 1.29	2.39 ± 0.78	2.12 ± 0.95	1.56 ± 1.01		
significance			III / V $p = 0.0307$				
Spreading,	W	L	LH				
$\bar{x} \pm SD$	2.05 ± 1.09	2.04 ± 0.81	2.78 ± 0.44				
significance		L / LH $p = 0.0175$					
Anat Loc,	Head	Trunk	Arm	Leg	Foot		
$\bar{x} \pm SD$	2.92 ± 0.29	2.33 ± 0.83	0.00 ± 0.00	2.16 ± 1.01	2.75 ± 0.50		
significance	H / L $p = 0.0339$		A / L $p = 0.0000$				
	H / A $p = 0.0000$		A / F $p = 0.0000$				
	H / T $p = 0.0235$		A / T $p = 0.0000$				
Patients,	Naevus	Dysplasia	Melanoma				
$\bar{x} \pm SD$	0.333 ± 0.483	0.381 ± 0.498	2.127 ± 0.952				
significance	N / Mel $p = 0.0000$	D / Mel $p = 0.0000$					

**Mitoses – number of mitoses; Ly Infiltr – lymphocyte infiltration; Clark – Clark score; Spreading-W – without spreading; L – lymphatic way of spreading; LH – lymphatic and hematogenous way of spreading; Anat Loc – Anatomical localisation.**

MW test) and pT4a ( $p = 0.0065$ , MW test) stages (Figure 2b). Melanomas with the highest number of mitoses (> 6) had a significantly higher intensity of VEGF expression in comparison with melanomas with no mitosis ( $p = 0.0200$ ), with 1 mitosis ( $p = 0.0147$ ) or with 2 mitoses ( $p = 0.0319$ ; MW test) (Figure 2c).

The highest average value of VEGF expression was detected in the melanomas without lymphocyte infiltration, and the minimum value was detected in the group with brisk lymphocytic infiltration. The difference between these two groups was statistically significant ( $p = 0.0245$ , MW test) (Figure 2d).

There was a significant difference in the intensity of VEGF staining in relation to the Clark level (Figure 2e). Melanomas Clark's level III had a significantly higher intensity of VEGF staining than melanomas Clark's level V ( $p = 0.0307$ ; MW test).

Average values of intensity of VEGF staining were highest in melanomas with lymphatic and hematogenous invasion. In addition, we found a significant difference in the intensity of VEGF staining between melanomas with only lymphatic invasion and those with both lymphatic and vascular invasion ( $p = 0.0175$ ; MW test) (Figure 2f).

Melanomas were divided into groups according to the anatomical localization of the primary tumor (Table 1). The highest average intensity of VEGF staining was detected in melanomas in the skin of the head ( $2.92 \pm 0.29$ ) (Table 2). This intensity of VEGF staining in the head region was

significantly higher than in the melanomas of the trunk or extremities (Table 2). Interestingly, after further substratification of melanomas arising in the skin of extremities on the arm, foot and leg, there was complete absence of VEGF staining in the melanomas of the arm (Figure 2g).

As we expected the average value of the tissue expression of VEGF was significantly higher in the samples of the melanoma patients in comparison to the average value of VEGF in the patients with nevi or dysplastic nevi ( $p < 0.0000$ ; MW test) (Figure 2h).

There was no statistically significant difference between the intensity of VEGF staining and the following parameters: growth phase, histological type of tumor, Breslow index, the presence of vascular invasion, regression and ulceration, and the presence of metastatic disease.

## Discussion

Our results show that the average value of the intensity of VEGF staining was significantly higher in melanoma ( $2.127 \pm 0.952$ ) compared to benign naevi ( $0.333 \pm 0.483$ ) and dysplastic naevi ( $0.381 \pm 0.498$ ). We found that 5 out of 81 patients with melanoma (2 in stage I, 2 in stage II and 1 in stage III) were completely negative to VEGF (5.7%). In the study of Konstatina et al.<sup>28</sup> from 2011, 13.69% patients with melanoma were negative for VEGF, but, in contrast with our control group results, 75% of patients with dysplastic nevi

had weak VEGF staining. The opinions on VEGF positivity in benign melanocytic lesions are divided. Some authors consider that the intensity of positivity depends on the nature of melanocytic lesions and that benign lesions have a low level of VEGF expression in comparison to the changes in malignant melanocytic lesions<sup>29</sup>. Pisacane and Risio<sup>30</sup> reported positive VEGF staining in 91% of their analyzed naevi and this was in contradiction with findings of Simonetti et al.<sup>31</sup> who did not find any VEGF positivity in benign melanocytic naevi, atypical melanocytic naevi or Spitz naevi. Some authors consider that the intensity of positive staining depended on the nature of the melanocytic lesions and that benign lesions have a low level of VEGF expression in comparison to the changes in malignant melanocytic lesions<sup>29</sup>. Such variations in immunohistochemical staining for VEGF may be explained by different clones of primary antibodies, antigen retrieval techniques, labelling systems and chromogens used in various studies. The most important of all is probably the difference in tissue fixation and processing<sup>32</sup>.

Our most interesting finding is the high intensity of VEGF staining in 90% of melanomas in clinical stage I, a finding that was expected in more advanced stages. Some researchers associated increased expression of VEGF in melanoma cells with tumor transition from horizontal to vertical growth phase<sup>33,34</sup>. As melanomas in only 6 out of 22 patients in clinical stage I (27%) and in 1 patient out of 18 in clinical stage II (5%) were in the horizontal phase of growth, we can conclude that angiogenic switch and locally enhanced synthesis of VEGF has already occurred. These results would indicate that in the early stage, small melanomas secrete and/or induce local production of VEGF, priming the tumor's micro-environment for subsequent growth, invasion and metastasis.

The highest percentage of patients with histological stage pT1a demonstrated greatest intensity of VEGF staining, whereas the maximum value of the average level of VEGF staining was detected, in decreasing order, in patients with stage pT2a, pT3b and pT4b. In the study of Gajanin et al.<sup>11</sup> the most intensive staining for VEGF was detected in patients with pT3b / pT4b histological stage. They found a significant difference in the intensity of expression of VEGF staining in patients with melanomas in different pT stages ( $p < 0.05$ ). They stated that the higher the pT stage, the higher the levels of VEGF expression (scores 2 and 3). Discrepancies between Gajanin's and our results could be explained by the greater average thickness of the tumor in the Gajanin et al.<sup>11</sup> series (7.09 mm vs 4.34 mm), by a smaller number of melanomas in the vertical growth phase, and by a difference in percentages of specific histological types of melanoma. In their study, nodular melanomas were present in 80% of cases, while in our study 23% of melanomas were nodular melanomas. Furthermore, they found that 15% of all melanomas were of the superficial spreading type and 5% of the lentigo maligna type, while our study showed 73% and 4%, respectively.

The presence of large numbers of mitotic figures is a sign of high metabolic activity, potential for tumor growth and metastatic spread. We found that the average intensity of VEGF staining in primary melanomas was in melanomas with the highest mitotic activity of  $> 6$  mitoses. The differ-

ence in intensity of VEGF staining in melanomas with the highest number of mitoses ( $> 6$ ) and those with no mitotic activity was statistically highly significant ( $p = 0.0200$ ), in melanomas with 1 mitosis ( $p = 0.0147$ ), and in melanomas with 2 mitoses ( $p = 0.0319$ ). Our results correlate well with the results of Gajanin et al.<sup>11</sup>.

Tumor-infiltrating lymphocytes (TIL) are a host immune response to melanoma cells, and use of this parameter is the accepted and widely used prognostic parameter for patients in clinical stage I melanoma<sup>35</sup>. There is evidence that melanomas induce specific humoral and cellular immune responses. Our results show that VEGF values were highest in melanomas without tumor infiltrating lymphocytes, and lowest in melanomas with the highest degree of tumor infiltrating lymphocytes ( $p = 0.0245$ ; MW test).

We found that the highest average intensity of VEGF staining was in melanomas Clark level III, and the lowest in melanomas Clark level V ( $p = 0.0307$ ). Rajabi et al.<sup>19</sup> found that melanomas with higher intensity of VEGF staining exhibited deep dermal invasion. In the same study, the comparison between the intensity of VEGF staining in melanomas with Clark's level showed that all patients with invasion of the reticular dermis and subcutaneous adipose tissue (Clark IV and V) had intensity of VEGF +2 and +3. The average Breslow thickness in their study was 1.84 mm. Gajanin et al.<sup>11</sup> found no statistically significant difference between the intensity of VEGF staining in the tumor and Clark level of invasion.

Our results show that the average value of intensity of VEGF staining was highest in melanomas with lymphatic and hematogenous invasion and comparison with the intensity of VEGF staining in melanomas without lymphatic and hematogenous invasion we found a statistically significant difference ( $p = 0.0175$ , MW test). We also found a positive correlation between the increase in the intensity of VEGF staining and disease progression.

A correlation of the intensity of VEGF staining in melanomas with its anatomical localisation show that the highest average value of the intensity of VEGF staining was detected in patients with melanoma localized in decreasing order in the head, foot, trunk and leg. In melanomas located on the skin of the hand there was no VEGF staining. Previous publications reported a good correlation between anatomical localization and different paths for tumor progression, and it was suggested that this was influenced by various etiologic factors such as sex, history of multiple serious sunburns, number of nevi, hairs bearing skin or not, the number of normal melanocytes in the particular skin localization and the presence of mast cells<sup>36-40</sup>. For example, the highest density of melanocyte was found to be in the skin of the head that contains hair follicles and is constantly exposed to UV radiation. The next localization with a slightly lower number of melanocytes was in the skin of the extremities and trunk<sup>36</sup>. It is generally accepted that melanoma in men is more frequent in the head and neck region than on the trunk. In women the most commonly affected region with melanoma is the skin of the lower extremities. This difference in melanoma localization between males and females could be explained by differences in the maximal sun exposure of dif-

ferent body parts in these two groups<sup>15</sup>. VEGF has been shown to play the important role in the biology of hair<sup>41</sup>. It was also found that the peritumoral accumulation of mast cells and, consequently, their release of potent angiogenic factors (including VEGF) leads to stimulation of angiogenesis and tumor progression<sup>37</sup>. According to the study of Callender et al.<sup>15</sup> patients with melanoma of the head and neck have increased synthesis of VEGF and worse prognosis than patients with skin melanomas in other localizations. This is related to the higher number of hair follicles, continuous exposure to UV radiation and a larger number of melanocytes and mast cells in the skin of the head. It is believed that, as the epidermal layer of the skin of the foot is thick, the melanomas in this location more frequently exhibit early vertical growth pattern and invasion into the deeper skin layers. This allows the tumor to grow to a considerably bigger size prior to any visible manifestations on the skin. Relatively high levels of VEGF together with relatively poor prognosis in trunk melanoma may be associated with multiple lymphatic drainage routes<sup>42</sup>. It is believed that patients with melanoma of the upper extremities have the best survival rate as they are more visible in this location than in others. Therefore, upper extremity melanomas draw appropriate medical attention while still in earlier stages<sup>37</sup>. In our study, only 4 patients had melanomas localized on the arm, and all of them were in the earliest stage, a possible explanation for the absence of VEGF

staining (score 0). Gajanin et al.<sup>11</sup> found the highest average intensity of VEGF staining in patients with melanoma of the extremities (2.36), followed by melanoma of the trunk (1.81) and melanoma of the head or neck (1.58). The above discrepancy between our study and that of Gajanin et al.<sup>11</sup> could be explained by a smaller experimental group (39 vs 81 patients in our study) of the latter, and a greater number of non-invasive, thin melanomas with horizontal growth phase of the same.

### Conclusion

We did not find a statistically significant difference between the intensity of VEGF staining and the following parameters: growth phase, histological type of tumor, Breslow index, the presence of vascular invasion, regression and ulceration, and the presence of metastatic disease. Our results indicate that VEGF plays the important role in the earliest stage of melanoma invasion, in further local tumor progression and in lymphovascular invasion and subsequent metastatic spread. The absence of VEGF expression in melanomas with brisk lymphocytic infiltrate was statistically significant. We conclude that the expression of VEGF in primary skin melanomas plays the important role in tumor progression and that more detailed studies must be done on VEGF prognostic value in melanoma on a larger number of patients.

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## Quantification of mast cells in different stages of periodontal disease

### Kvantifikacija mastocita u različitim stadijumima parodontalne bolesti

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#### Abstract

**Background/Aim.** Mast cells are mononuclear cells originating from bone marrow. They produce various biologically active substances, which allow them to actively participate in immune and inflammatory processes associated with periodontal disease. The study focused on distribution and density of mast cells in healthy gingiva as well as in different stages of periodontal disease. **Methods.** The material used for this purpose was gingival biopsies taken from 96 patients classified into 4 groups: healthy gingiva, gingivitis, initial and severe periodontal disease. Toluidine blue staining according to Spicer was utilized for identifying mast cells. **Results.** Basing on our study, the density of mast cells in the gingival tissue increases with the progression of the infection, which means they are more numerous in gingivitis compared to healthy gingiva, as well as in periodontal disease compared to gingivitis. **Conclusion.** Increase in the number of mast cells in the infected gingiva can be correlated with an increased influx of inflammatory cells from blood circulation into the gingival stroma, as well as with the collagen lysis, since these cells produce substances with collagenolytic potential. Based on the distribution of mast cells, it could be concluded that in the evolution of periodontal disease there are significant dynamic alterations in migration and localization of these cells.

**Key words:** periodontal diseases; disease progression; mast cells.

#### Apstrakt

**Uvod/Cilj.** Mastociti su mononukleusne ćelije poreklom iz koštane srži. Oni proizvode brojne biološki aktivne supstance, usled čega aktivno učestvuju u imunim i inflamatornim procesima kod parodontalne bolesti. U istraživanju je praćena distribucija i gustina mastocita u zdravoj gingivi i u različitim fazama parodontalne bolesti. **Metode.** Materijal je dobijen gingivalnom biopsijom kod 96 bolesnika razvrstanih u 4 grupe: zdrava gingiva, gingivitis, početna parodontopatija i uznapredovala parodontopatija. Za identifikaciju mastocita korišćeno je toluidin plavo bojenje po Spiceru. **Rezultati.** Prema našem istraživanju gustina mastocita u gingivalnom vezivu raste sa progresijom infekcije, što znači da su mastociti u gingivitisu brojniji u odnosu na zdravu gingivu, a u parodontopatiji brojniji u poređenju sa gingivitisom. **Zaključak.** Povećanje broja mastocita u inficiranoj gingivi može se dovesti u vezu sa pojačanim inluksom inflamatornih ćelija iz cirkulacije u stromu gingive, ali i sa lizom kolagena budući da ove ćelije proizvode supstance sa kolagenolitičkim potencijalom. Na osnovu distribucije mastocita može se zaključiti da u evoluciji parodontalne bolesti postoje značajne dinamičke alteracije u migraciji i lokalizaciji ovih ćelija.

**Ključne reči:** periodontalne bolesti; bolest, progresija; mast ćelije.

#### Introduction

Periodontitis or periodontal disease is a chronic inflammatory disease associated with bacterial infection<sup>1</sup>. The disease is characterized by destruction of the periodontal ligament and gingiva and by alveolar bone loss<sup>2</sup>. Periodontal disease goes through various stages – gingivitis, initial and severe periodontal disease<sup>3</sup>. Periodontal disease is one of the

most common diseases nowadays, and at the same time it is a risk factor for various systemic diseases such as diabetes, cardiovascular, renal or pulmonary disease<sup>4</sup>. Periodontal pathogens and their products cause pathological changes, but cannot contribute to the comprehensive development of periodontal disease<sup>5</sup>. It has been suggested that the reaction of the patient's immune system has a key role in the destruction of periodontal tissues<sup>6</sup>. Numerous types of cells, including

mast cells with their important role are involved in the pathogenesis of periodontal disease.

Mast cells (MCs) are mononuclear cells originating from CD34+ precursors from bone marrow<sup>6</sup>. They differentiate and mature in peripheral tissues<sup>7</sup>. MCs are normal residents of the *lamina propria* of human oral mucosa and gingiva<sup>8</sup>. They produce various biologically active substances, which allow them to actively participate in immune and inflammatory processes associated with periodontal disease<sup>9</sup>. In their cytoplasm, they contain about 80 to 300 granules which exhibit metachromasy and toluidine blue staining<sup>10</sup>. Various enzymes and mediators, such as the serine proteases, tryptases, chymases, cathepsin G, acid hydrolases, matrix metalloproteinase, histamine, heparin and serotonin are stored in granules<sup>11</sup>. Based on the content of neutral serine proteases human MCs can be divided into mast cell tryptases (MCT) and mast cell tryptase and chymotryptic proteinase (MCTC). MCTC in their granules contain tryptases, chymases, carboxypeptidases and cathepsin G-like proteases, and mainly can be found in the dermis and intestinal submucosa<sup>12</sup>. Of neutral proteases, MCT contain only tryptases and primarily can be found in red mucous membrane and in alveolar walls<sup>13</sup>. MCs release proinflammatory mediators, promote inflammation and angiogenesis, degeneration of the extracellular matrix and tissue remodeling<sup>10</sup>. These cells are noted in different parts of gingiva, and information on their density in healthy and inflamed tissues is contradictory. The aim of the study was to examine the distribution and density of various stages of periodontal disease in order to assess the importance of this cell population in a specific immune response and related tissue destruction.

## Methods

The research was undertaken at the Dental Clinic and Institute of Histology of Faculty of Medicine in Kosovska Mitrovica. This research has been conducted following the principles of the Declaration of Helsinki (retrieved from <http://www.wma.net/en/30publications/10policies/b3/>. Last accessed: 20-12-2014) and approved by the Institutional Review Board of the Faculty of Medicine in Kosovska Mitrovica. Informed consent was issued by all patients after a careful explanation of the aims of the study.

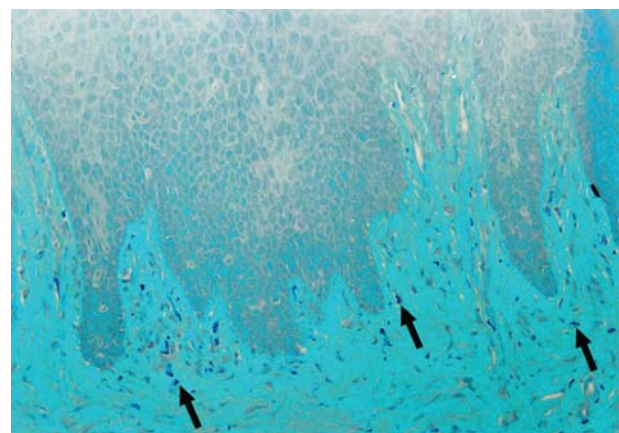
The material consisted of gingival biopsies taken from 96 patients aged 13–68 years. Biopsies were made during extractions of teeth for orthodontic reasons or periodontal disease. Patients included in this study did not suffer from systemic diseases. The following indices: Community Periodontal Index of Treatment Needs (CPITN), Löe-Silness gingival index, Mühlemenn-Sulcus bleeding index, Silness-Löe plaque index were used to assess the clinical stage of the disease. Periodontal pocket depth was measured with a ball tip probe. In accordance with the classification system for periodontal diseases and conditions of the American Academy of Periodontology<sup>14</sup>, gingival samples were classified into four main groups: healthy gingiva (11 samples taken from people whose gingiva showed no signs of bleeding, swelling or inflammation, and where gingival groove depth was usual);

gingivitis (18 tissue samples taken from patients reported with redness, swelling and bleeding gums, and Löe-Silness gingival index was 2 or 3); moderate periodontitis (36 samples taken from patients with periodontal pocket depth below 6 mm); severe or advanced periodontitis (31 samples taken from patients with periodontal pocket depth over 6 mm).

Tissue samples were fixed in 10% formalin, dehydrated in increasing concentrations of alcohol, lightened in xylene and embedded in paraffin. Serial sections of 5 mm thicknesses were made from the paraffin blocks using Reichert sliding microtome. Spicer's staining technique was used for identification of mast cells<sup>15</sup>. Morphometric analysis was done using previously calibrated mesh whose surface was 0.0145 mm<sup>2</sup> at 400× microscope magnification. For each measurement, the mesh was put over the entire tissue sample, whose surface required a certain number of measurements *per* sample. The average number of cells *per* mesh surface was determined for each sample and the number of cells *per* mm<sup>2</sup> of gingival tissue is obtained with its dividing by 0.0145. Statistical analysis was done using MS Office Excel program. The density of MCs *per* unit area (mm<sup>2</sup>) was expressed in mean value ± standard deviation. Testing the statistical significance of differences in the mean values of the number of MCs *per* mm<sup>2</sup> of gingival tissue between the experimental groups was done using Student's *t*-test.

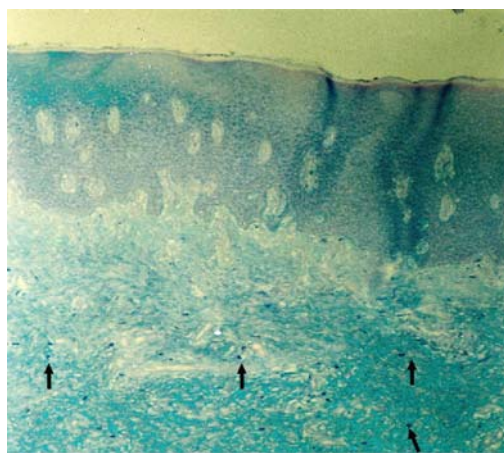
## Results

A moderate number of round, oval or spindle-shaped MCs can be seen in preparations of healthy gingiva (Figure 1). They can be found as single cells, rarely in smaller groups. MCs were more numerous in the reticular than in papillary layer of gingiva, and most of them were on the border between the two layers and along blood vessels. MCs cannot be found in gingival epithelium. In gingivitis, it is reported that there is an increased number of MCs in reticular gingival layer with the tendency to clustering around arterial and venous blood vessels, around inflammatory infiltrates and within them, whereas they are very rare and usually oblong-shaped between densely organized collagen bundles (Figure 2).

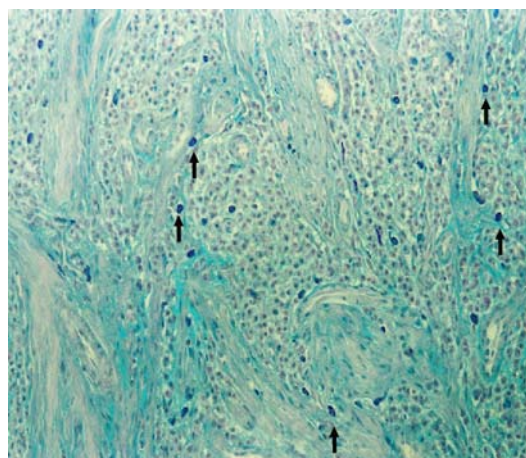


**Fig. 1 – Mast cells (black arrows) in healthy gingiva were most numerous on the border between the reticular and papillary layer (Alcian blue, 200 ×).**

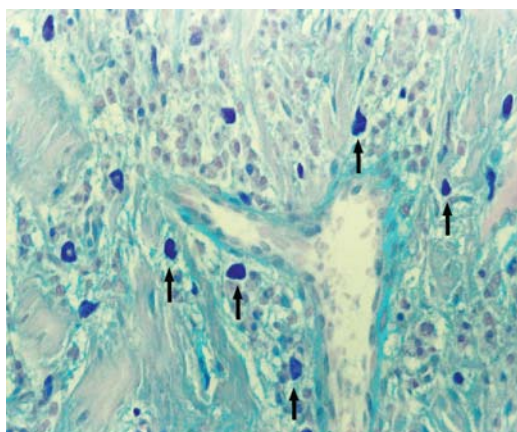
No significant differences were found in the density and distribution of MCs between moderate and advanced periodontitis, but compared to healthy gingiva and gingivitis, a noticeable increase in their number was reported in both groups. In periodontal disease, MCs were plentiful in collagen bundles and inflammatory foci (Figure 3), but it is evident that most of them were around inflammatory infiltrates and blood vessels (Figure 4). It was notable that MCs within infiltrates were round-shaped and to some extent degranulated, whereas MCs along collagen bundles were by the rule oblong-shaped. In periodontal disease, mast cell density was increased in papillae and usually at their base, rarely at the top. MCs in the basal and spinous layers of the oral gingival epithelium were observed in two samples with initial periodontal disease and in three samples with advanced periodontal disease (Figure 5).



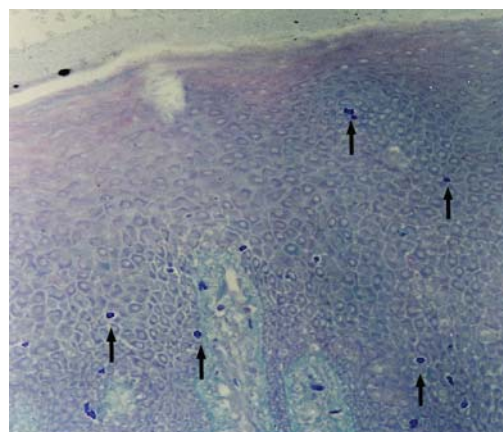
**Fig. 2** – In gingivitis, mast cells (arrows) were densest in the reticular layer, much rarer in the papillary layer of gingiva and not found in the epithelium (Alcian blue, 100 ×).



**Fig. 3** – In periodontal disease mast cells were particularly numerous on the border of inflammatory foci with collagen bundles (arrows) (Alcian blue, 200 ×).



**Fig. 4** – A lot of mast cells (arrows) surrounding a blood vessel in advanced periodontal disease (Alcian blue, 400 ×).



**Fig. 5** – In some samples of periodontitis mast cells (arrows) were found in the gingival epithelium (Alcian blue, 200 ×).

By doing stereological study and statistical analysis it was found that the binder in normal gingiva contained an average of  $59.43 \pm 19.85$  mast cells/mm<sup>2</sup>, in gingivitis  $96.83 \pm 25.72$  cells/mm<sup>2</sup>, in initial periodontal disease  $136.42 \pm 28.52$  cells/mm<sup>2</sup>, in advanced periodontal disease  $128.43 \pm$

$42.89$  cells/mm<sup>2</sup>. The average number of MCs in the group with initial periodontal disease was statistically significantly higher than in healthy gingiva ( $p < 0.001$ ) and gingivitis ( $p < 0.05$ ). Both gingivitis group ( $p < 0.05$ ) and advanced periodontal disease group ( $p < 0.01$ ) had a higher density of MCs compared to healthy gingiva. The difference in the average number of MCs was not statistically significant between the initial and advanced periodontal disease.

## Discussion

MCs secrete primary and secondary inflammatory mediators and have an important role in inflammatory reactions<sup>10</sup>. In our study, detection of MCs was performed using Alcian blue polyvalent basic dye according to Spicer's method. The results suggest that MCs are dispersed throughout

the binder of healthy gingiva, mostly as single cells, sometimes in smaller groups. Naesse et al.<sup>11</sup> studied the number of MCs in three randomly selected gingival zones (the zone beneath marginal epithelium, the central zone and the zone beneath oral epithelium) and found no differences in the

density of MCs. The same authors recorded a number of MCs in the gingival epithelium as well. In our material, MCs were found neither in epithelium of healthy gingiva, nor in gingivitis, but in some samples of moderate to advanced periodontal disease they were found in oral epithelium. Similar results were recently published by Popovici et al.<sup>16</sup>. These authors found that the number of intraepithelial MCs increases with the severity of inflammation and intraepithelial MCs could be important in the evaluation of the severity of periodontal disease.

Some authors have recorded a connection between MCs and peripheral nerves which secrete neuropeptides (substance P, vasoactive intestinal peptide). It is well-known that neuropeptides can induce degranulation of MCs<sup>17</sup>, and thus affect the course and severity of inflammatory reactions. Huang et al.<sup>18</sup> pointed to the correlation between MCs degranulation and the severity of periodontal disease. In our study, it has been recorded that MCs can often be found on the border of inflammatory infiltrates. Previously, Mekori and Metcalfe<sup>19</sup> recorded similar findings in their studies. They noticed MCs surrounding T-lymphocytes and concluded that it is possible that these cells phagocytize, process and present antigens to T-lymphocytes thus initiating adaptive immune response<sup>20</sup>. In addition, there is proof that MCs secrete both Th1-type and Th2-type cytokines, suggesting that these cells may send an immune response to an inflammatory or anti-inflammatory reaction. It is known that MCs secrete a significant amount of IL-4. Thus favoring the Th2 immune response followed by activation of B-lymphocytes and production of antibodies. Previous observations confirm the existence of functional relations between MCs and immunocompetent cells. Batista et al.<sup>21</sup> observed that MCs are concentrated in a large number not only in the inflammatory focus, but also around it. They recorded that in localized chronic periodontitis the number of MCs was increased by 3% around the inflammatory focus and by about 28% in the focus itself. Quantification of MCs within and around the inflammatory focus was not done in our study, but in many samples of periodontal disease a larger number of these cells were found around the focus than within it. These results suggest that there are significant dynamic alterations in migration and localization of MCs in the evolution of periodontal disease.

It is known that MCs start to develop in bone marrow, then enter the peripheral circulation and mature in tissues. MCs of connective tissue have a long lifetime and require stem factors for their development and survival<sup>22</sup>. Mucosal MCs mature in mucosa of various organs after exposure to Th2-type anti-inflammatory cytokines and their number increases in the case of parasitic infection or allergy<sup>22</sup>. Since MCs do not proliferate locally, their precursors come into gingiva from bone marrow, and thus it can be possible to conclude that there are certain processes that direct MCs precursors to the inflammatory focus<sup>23</sup>.

In the literature, there is a strong disagreement about the number of MCs in healthy and unhealthy gingiva. While some authors have recorded an increase in the number of MCs in periodontal disease<sup>24</sup>, others have recorded a

decrease in their number<sup>25,26</sup>. Cindrić et al.<sup>27</sup> have recorded an increase in the number of MCs in inflamed gingiva in patients with gingival index 1–2 and a decrease in their number in patients with gingival index 3. According to our research, the binder in normal gingiva contains an average of  $59.43 \pm 19.85$  mast cells/mm<sup>2</sup> of binder, in gingivitis  $96.83 \pm 25.72$  cells/mm<sup>2</sup>, in initial periodontal disease  $136.42 \pm 28.52$  cells/mm<sup>2</sup> and in advanced periodontal disease  $128.43 \pm 42.89$  cells/mm<sup>2</sup> of binder. The average number of MCs in healthy gingiva was significantly lower compared to the other tested groups, and in gingivitis was significantly lower than in both forms of periodontal disease. On the other hand, no significant difference was noted in the average number of MCs between initial and advanced periodontal disease. In 1950, Wislocki and Sognnaes<sup>28</sup> reported that the number of MCs *per mm*<sup>2</sup> of healthy gingival tissue ranged from 30 to 60 cells, which is equivalent to our findings, while by doing quantitative analysis, Dummett et al.<sup>29</sup> concluded that the number of MCs in gingiva ranged from 0.47 to 146 cells per mm<sup>2</sup>. Batista et al.<sup>21</sup> also performed quantification of MCs in various stages of periodontal disease on preparations stained with toluidine blue. According to their findings, healthy gingival tissue contained an average of  $35.73 \pm 37.77$  mast cells/mm<sup>2</sup>, in gingivitis that number increased to  $44.54 \pm 30.31$  cells/mm<sup>2</sup>, whereas in the localized chronic periodontitis their density was the highest ( $71.38 \pm 59.15$  cells/mm<sup>2</sup>). In comparison with our results, it is evident that there is a lower cell density (which can be attributed to different staining techniques), but the trend of increasing cell density is consistent to disease progression.

The increase in the number of MCs in acute and chronic inflammation of gingiva explains the role of these cells in the pathogenesis of the disease. There are results that confirm the correlation between the density of MCs in tissues and hyperemia, as well as the connection between increased vascular permeability and degranulation of MCs accompanied with histamine release<sup>30</sup>. Since MCs serve as a major source of histamine in tissues<sup>31</sup>, the increase in their number can be related to an increased influx of leukocytes into the inflammatory focus.

The results by Naesse et al.<sup>11</sup> showed that of all cells of gingival infiltration, MCs express matrix metalloproteinase (MMPs) most prominently. They concluded that the concentration of MCs that express MMPs increases in periodontal disease. Since these enzymes are responsible for the destruction of collagen, it is possible to make conclusions about the participation of MCs in both defense mechanisms and destructive processes during chronic inflammation of periodontal tissues. The destructive effect may be of IL-4 originating from MCs, which directs the immune response towards the production of antibodies, which happens in exacerbations. Further, tryptases also have collagenolytic potential and may contribute to the tissue destruction in periodontal disease<sup>9</sup>. The byproducts of MCs could be correlated with bone resorption since the deficit of MCs is associated with lower levels of bone remodeling, and excess of MCs could lead to acceleration of bone turnover<sup>32</sup>.

## Conclusion

According to our study, the density of mast cells in the gingival binder increases with the infection progression, so these cells are more numerous in periodontal disease as compared to gingivitis and healthy gingiva, and in gingivitis they are more numerous than in healthy gingiva.

No differences in the density of mast cells were found between initial and advanced periodontal disease. The incre-

ase in the number of mast cells in the infected gingiva may be the consequence of the need for increased migration of inflammatory cells from blood circulation into gingival stroma, but it can be correlated with collagen lysis since these cells produce matrix metalloproteinase and tryptases. By inspecting the distribution of mast cells, it can be possible to conclude that there are significant dynamic alterations in migration and localization of these cells in the evolution of periodontal disease.

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# Long-term motor and sensory outcomes after surgery for infantile esotropia

## Dugoročni motorni i senzorni rezultati operacije dečje ezotropije

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### Abstract

**Background/Aim.** Infantile esotropia (ET), entitled as congenital ET, is defined as an alternating, cross-fixational ET that occurs within the first 6 months of life. The aim of this study was to determine the long-term motor and sensory outcomes after surgical correction of patients with infantile ET. **Methods.** Medical records of 108 consecutive children who had bimedial rectus recession (BMR) initially for ET were reviewed retrospectively. The patients were divided into 3 groups: the group I, surgery before one-year old; the group II, surgery between one and two-year old; the group III, surgery after two-year old. **Results.** No significant differences were determined among the groups for preoperative mean angle of deviation and refractive error ( $p > 0.05$ , for both). Development rate of dissociated vertical deviation (DVD) was greater (40%) in the group I, and the relationship between the rate of DVD and the timing of the initial surgery was statistically significant ( $p = 0.03$ ). Risk for additional surgery was significantly greater in patients with a younger mean age at initial surgery ( $p = 0.01$ ). Although measurable stereopsis rate was higher in the group I (35%, 32.4%, 27.8%, respectively) the difference among the groups was insignificant ( $p = 0.80$ ). **Conclusion.** Patients with ET have limited potential of high grade stereoacuity despite the early alignment of eyes. Early surgery also has potential effects for the development of both inferior oblique overaction and DVD earlier.

### Key words:

esotropia; infant; child, preschool; ophthalmologic surgical procedures; treatment outcome.

### Apstrakt

**Uvod/Cilj.** Dečja ezotropija (ET), koja se naziva i urođena ET, definiše se kao naizmenična, poprečnofiksirajuća ET koja nastaje tokom prvih šest meseci života. Cilj ove studije bio je da se utvrde dugoročni motorni i senzorni rezultati operacije mališana sa dečjom ezotropijom. **Metode.** Urađen je retrospektivni uvid u medicinsku dokumentaciju 108 ET dece sa bimedijalnom pravom recesijom (*bimedial rectus recessio* – BMR). Izvršena je podela na tri grupe: grupa I – operacija pre uzrasta od jedne godine, grupa II – operacija između jedne i dve godine i grupa III – operacija posle dve godine života. **Rezultati.** Nisu nađene značajne razlike među grupama u odnosu na preoperativni srednji ugao devijacije i refraktivnu grešku ( $p > 0,05$  za oba parametra). Stopa razvoja disocijacije vertikalne devijacije (DVD) bila je viša (40%) u grupi I, a veza između stope DVD i vremena prve operacije bila je značajna ( $p = 0,03$ ). Rizik od dodatne operacije bio je veliki kod dece prvobitno operisane u ranijem srednjem dobu ( $p = 0,01$ ). Iako je merljiva stopa stereopsisa bila viša u grupi I (35%; 32,4%; 27,8%, redom), razlika među grupama bila je beznačajna ( $p = 0,80$ ). **Zaključak.** Kod dece sa ET mala je mogućnost značajne oštine steroskopskog vida i pored ranog usklađivanja očiju. Rana operacija može da ima uticaja i na razvoj oba ova poremećaja.

### Ključne reči:

ezotropija; odojče; deca, predškolska; hirurgija, oftalmološka, procedure; lečenje, ishod.

## Introduction

Infantile esotropia (ET), entitled as congenital ET, is defined as an alternating, cross-fixational ET that occurs within the first 6 months of life<sup>1</sup>. Various abnormal sensory and motor disturbances such as asymmetric optokinetic nystagmus, abnormal stereopsis, dissociated vertical deviation (DVD), inferior oblique overaction (IOOA) and latent nystagmus can be exhibited by time<sup>2</sup>. Although infantile ET has been classically described as a large-angle constant ET, amount of deviation may alter and accommodative component might be added in time<sup>3</sup>. Its aetiology has not precisely been known.

Although the most applied treatment option in infantile ET is bilateral medial rectus recession (BMR) surgery, the most beneficial timing of surgery remains controversial<sup>4</sup>. Infantile ET is generally associated with a poor binocular visual outcome<sup>2</sup>. While early (within one year)<sup>1</sup> and very early (within 6 months)<sup>5</sup> surgeries ensures better sensory results<sup>4</sup>, delayed surgery may provide better orthophoria and keeps away multiple interventions<sup>6,7</sup>.

The aim of this study is to present late sensory and motor outcomes of BMR surgery for the treatment of infantile ET.

## Methods

Data of 108 patients who had BMR surgery for infantile ET and were followed for at least three years at the ophthalmology department of our institution between 1989 and 2011 were retrospectively reviewed. Approval from Institutional Review Board was obtained and the tenets of the Declaration of Helsinki was observed.

Inclusion criteria for patients consisted of constant ET of 25 prism diopters (PD) or more beginning before 6 months of age,  $\leq +3.50$  diopters (D) refractive error. Exclusion criteria were: gestational age  $\leq 34$  weeks, congenital nystagmus, neurologic defects, developmental delay, dysmorphia, birth trauma, paralytic strabismus, and preoperatively determined development of DVD and/or IOOA.

The preoperative and follow-up ocular examinations were performed including evaluation of visual acuity, refractive error with cyclopentolate (Sikloplejin<sup>®</sup>, Abdi Ibrahim, Istanbul, Turkey), and a complete eye examination including ophthalmoscopy and ocular motility. Visual acuity (VA) was tested with a linear tumbling "E" or Snellen charts in verbal children and with the presence or absence of normal fixation in preverbal children. Pre- and postoperative angle of deviations (preoperative measurements were those immediately prior to surgery and not the deviation size at initial presentation) at near (30 cm) and distance (5 m) were measured by prism cover test with appropriate spectacle correction in primary, gaze up and gaze down positions when possible. If this was not possible, angle of deviation was measured with corneal light reflections (Hirschberg method) or Krimsky prism tests. Ductions and versions were evaluated by both observation and photographic recording. Inferior oblique muscle overaction or underaction, and DVD were examined. Stereoacuity was measured by the Titmus stereopsis test.

Surgeries were applied under general anesthesia by opening limbus-based conjunctival incision in all cases. Muscles were reattached to the sclera by using partial thickness scleral passes, and recession was measured from the original muscle insertion. Surgical dosages were determined according to standard tables<sup>8</sup>. Additional treatment was performed as needed for residual ET, secondary exotropia (XT), IOOA, DVD, accommodative ET, or amblyopia. Postoperative surgical success was defined as alignment within 10 PD of orthotropia at the final examination.

Patients meeting the abovementioned eligibility criteria were divided into 3 groups based on the time of the initial BMR surgery: the group I, surgery before one-year old; the group II, surgery between one and two-year old; the group III surgery after two-year old. Pre- and postoperative angle of deviation, additional extraocular muscle surgeries, the number of surgical procedures, VA, fixation preference, refraction, stereopsis, amount of the recession of the rectus muscles, development of IOOA and DVD were evaluated and the results of the three groups compared.

The normal distribution was checked using the Kolmogorov-Smirnov test. Comparisons between the groups were completed using a Pearson  $\chi^2$  test for categorical variables and a non-parametric Kruskal-Wallis test for continuous data. Non-parametric *post-hoc* tests with bonferroni correction were performed to datas which were significant with Kruskal-Wallis test. IBM SPSS Statistics for Windows Ver. 20.0 was used for the statistical analysis, and  $p$  value  $< 0.05$  was considered significant.

## Results

Pre- and postoperative data of the groups are summarized in Tables 1 and 2. Distribution of sex and mean follow-up time among the groups were similar ( $p > 0.05$ ). There was no significant difference among the groups in refractive error or angle of deviation at the initial visit ( $p > 0.05$ ). Visual acuity of the worse eye taken at the final examination was higher in the group III, but there was no statistically significant difference among the groups (VA of the worse eye was analyzed as a continuous variable  $p = 0.06$ ). The mean angle of ET at the last visit after the surgery was better in the groups II and III compared to the group I ( $p = 0.04$ ,  $p < 0.001$ ; respectively). The mean amount of medial rectus recession was similar in all the groups ( $p > 0.05$ ). Second-step or more surgery was performed on 65% children in the group I and 38.2% in the group II and 27.8% in the group III ( $p = 0.03$ ). The rate of consecutive XT after surgery was higher in the group I ( $p = 0.32$ ). Postoperatively, IOOA developed 50.9% of all cases by time and the difference between the rates of IOOA of the groups was insignificant ( $p = 0.62$ ). However, the mean age of the patients for the development of IOOA was significantly low with respect to the other groups in the early surgery group ( $p = 0.006$ ,  $p < 0.001$ ; respectively). The presence of IOOA was associated with a higher rate for the development of consecutive XT ( $p < 0.01$ ). DVD development was also higher in the group I and a significant difference was found among the groups ( $p = 0.03$ ).

Table 1

Preoperative data of the patients				
Patients	Group 1 (< 1-year-old)	Group 2 (> 1 and < 2-year-old)	Group 3 (> 2-year-old)	<i>P</i>
Number of patients (n)	20	34	54	
Gender (%)				
male	12 (60)	23 (68)	33 (61)	0.78
female	8 (40)	11 (32)	21 (39)	
Preoperative refractive error [D; mean ± SD (range)]				
spheric	1.7 ± 0.8 (0–3.25)	1.4 ± 1.3 (-3.25–3.25)	1.6 ± 1.1 (-1.00–3.25)	0.80
astigmatic	0.5 ± 0.8 (0–2.75)	0.4 ± 0.6 (-0.75–1.75)	0.5 ± 0.7 (-1.50–3.00)	0.67
Preoperative angle of deviation [PD; mean ± SD (range)]				
distance	32.7 ± 10.4 (25–70)	36.9 ± 11.5 (25–70)	34.3 ± 9.2 (25–70)	0.29
near	32.7 ± 10.4 (25–70)	37.0 ± 11.9 (25–70)	34.2 ± 8.8 (25–60)	0.36
Age at initial examination [months; mean ± SD (range)]	8.4 ± 3.5 (3–12)	15.6 ± 4.9 (8–24)	40.4 ± 22.6 (10–132)	< 0.0001* (The same for all comparisons)
Age at first surgery [months; mean ± SD (range)]	10.2 ± 2.1 (6–12)	19.6 ± 4.2 (13–24)	47.7 ± 20.9 (25–144)	< 0.0001* (The same for all comparisons)
Amount of BMR surgery [mm; mean ± SD (range)]	5.3 ± 0.3 (5–6)	5.5 ± 0.3 (5–6)	5.2 ± 0.3 (5–6)	Group 1–2 0.48 Group 1–3 0.48 Group 2–3 0.15

D – diopters; n – number; PD – prism diopters; SD – standard deviation; BMR – bimedian rectus recession.

\**p* < 0.05; †*Post hoc* tests with Bonferroni correction were applied to all datas which were significant with Kruskal Wallis Test.

Table 2

Postoperative data of the patients				
Characteristics	Group I (< 1-year-old)	Group II (> 1 and < 2-year-old)	Group III (> 2-year-old)	<i>P</i>
n	20	34	54	
Only BMR, [n (%)]	7 (35)	21 (61.8)	39 (72.2)	0.03*† (Significance results from Group I)
Additional surgery, [n (%)]	13 (65)	13 (38.2)	15 (27.8)	
<i>BMR+Residual esotropia surgery</i>	7 (35)	6 (17.6)	7 (13)	
<i>BMR+Inferior oblique muscle surgery</i>	1 (5)	3 (8.8)	6 (11.2)	
<i>BMR+Consecutive exotropia surgery</i>	4 (20)	3 (8.8)	1 (1.8)	
<i>BMR+DVD surgery</i>	1 (5)	1 (3)	1 (1.8)	
Number of operations [mean ± SD (range)]	1.95 ± 1.0 (1–4)	1.50 ± 0.75 (1–3)	1.35 ± 0.75 (1–5)	Group 1–2 0.24 Group 1–3 0.01* Group 2–3 0.78
Follow-up period [years; mean ± SD (range)]	7.75 ± 3.1 (3–13)	7.56 ± 4.0 (3–18)	6.87 ± 3.0 (3–16)	0.54
Refractive error at final visit [D; mean ± SD (range)]				
spheric	1.1 ± 1.2 (-1.50–3.75)	1.2 ± 2.0 (-7.00–4.75)	1.0 ± 1.2 (-1.00–5.00)	0.46
astigmatic	0.3 ± 1.6 (-4.50–3.00)	0.7 ± 1.1 (-1.75–3.25)	0.6 ± 1.0 (-2.00–4.50)	0.62
Angle of deviation at final visit [PD; mean ± SD(range)]				Group 1–2 0.04* Group 1–3 < 0.001* Group 2–3 1 0.09
distance	8.8 ± 5.0 (0–18)	5.7 ± 6.6 (0–25)	4.4 ± 5.2 (0–20)	
near	9.2 ± 5.1 (0–18)	6.7 ± 6.4 (0–20)	6.2 ± 5.4 (0–18)	
Stereopsis, [n (%)]				
present	7 (35)	11 (32.4)	15 (27.8)	0.80
absent	13 (65)	23 (67.6)	39 (72.2)	
Postoperative IOOA, [n (%)]	11 (55)	19 (55.9)	25 (46.3)	0.62
Age of the development of IOOA, [months; mean ± SD (range)]	22.9 ± 11.5 (8–42)	55.3 ± 33.0 (20–131)	54.8 ± 20.1 (18–111)	Group 1–2 0.006* Group 1–3 < 0.001* Group 2–3 1
Postoperative DVD, [n (%)]	8 (40)	6 (17.6)	7 (13)	0.03* (Significance results from Group I)
Age of the development of DVD [months; mean ± SD (range)]	43.3 ± 28.3 (24–108)	90.6 ± 47.5 (50–143)	77.0 ± 41.5 (24–128)	Group 1–2 0.03* Group 1–3 0.03* Group 2–3 0.51
Consecutive exotropia at the final visit [n (%)]	5 (25)	6 (17.6)	6 (11.1)	0.32
The VA of the worse eye at the final visit [mean ± SD (range)]	0.75 ± 0.32 (0.1–1.0)	0.77 ± 0.24 (0.1–1.0)	0.85 ± 0.23 (0.2–1.0)	0.06
Success rate [n (%)]				
distance	14 (70)	27 (79.4)	48 (88.9)	0.14
near	11 (55)	25 (73.5)	42 (77.8)	0.14

D – diopters; N – number; PD – prism diopters; SD – standard deviation; IOOA – inferior oblique muscle overaction; DVD – dissociated vertical deviation; VA – visual acuity; BMR – bimedian rectus recession.

\**p* < 0.05; †difference between only bimedia rectus recession (BMR) and additional surgery. *Post hoc* tests with Bonferroni correction were applied to all datas which were significant with Kruskal Wallis test.



The mean number of reoperation was smaller in the group III compared to the group I ( $p = 0.01$ ). Success rate (defined as deviation of  $\leq 10$  PD at the final examination) was higher in the group III, but it was not statistically significant ( $p = 0.14$ ).

The number of patients who had developed gross stereoaquity (at least 3000 seconds of arc) at the final visit was higher in the group I (median = 400 seconds of arc  $p = 0.80$ ). No patient had high-grade (40–60 sec of arc) stereopsis within the groups. No postoperative complications were observed in any of the patients. Anterior segment and fundoscopic examination were normal in all the patients.

## Discussion

The aim of the infantile ET treatment is to provide orthophoria with better binocular vision and ocular motor development. However, deficient binocular vision and maldevelopment of stereopsis are obtained<sup>2</sup>. Though some spontaneously recovered cases have been reported, main treatment for infantile ET is surgery<sup>9, 10</sup>. The proper time for surgery has been debated for decades, and there is growing evidence from clinical and animal studies describing enhanced sensory and ocular motor development after early surgery during the critical periods of development<sup>11</sup>. While main disadvantages of early surgery were increased risk of amblyopia and difficulty in obtaining reliable and accurate preoperative measurements, more reliable and accurate preoperative measurements were main advantages of late surgery<sup>3</sup>. However, a long-term study addressed that orthoptic measurement had no negative impact on long-term eye alignment in patients who had early surgery for infantile ET<sup>12</sup>.

Although infantile ET requires surgical correction, the most beneficial timing of surgery remains controversial. According to our results, it is clear that alignment can be achieved successfully in children with infantile ET with one surgical procedure performed after age one-year (Table 2). While 35% of the patients achieved a final ocular alignment within 10 PD with one operation in the group I, it was 52.8% of the patients who had surgery after one year of age ( $p = 0.03$ ). Common concerns about early surgery are that the angle of deviation may have greater instability and that it may be difficult to obtain accurate measurement of the angle of deviation for surgical planning<sup>12, 13</sup>. In our study there was no significant differences between the early and late surgery groups in the rate of postoperative surgical success. However, the rate of additional surgical procedures for ocular alignment of the early surgery group (65%) was significantly higher with respect to other groups ( $p = 0.03$ ). Wright et al.<sup>14</sup> performed additional surgery on 57% of the patients who had early surgical alignment and who were followed for 2–8 years. Birch et al.<sup>15</sup> did on average 1.5 surgeries on 73 patients followed 4 to 7 years and our results with a longer follow-up period (1.51 surgeries on 108 patients followed 3–18 years) were similar to those of Birch et al. Helveston et al.<sup>1</sup> reported 1.1 additional reoperation procedures rate per patient who had BMR surgery between 83 and 159 days of age and who were followed for 8 to 10 years. In the present study, additional operation rate per patient who had BMR surgery before one

year of age with 3–13 years follow-up was 1.6. Thus early surgical alignment of patients with infantile ET may not assure the maintenance of alignment.

It was reported that the consecutive XT ratio was higher (38%) in patients operated before 6 months of age compared to the patients operated after 6 months of age (26%) for infantile ET. However, all the patients were treated with 7 mm BMR surgery in that study<sup>16</sup>. Another factor that might have been responsible for developing consecutive XT may be larger recessions ( $> 6.0$  mm) in the early group, but maximum recession value was 6.0 mm in our study. Larger recessions of medial rectus muscles also may have an influence on the development of adduction limitation which is also a significant risk factor for the development of consecutive XT in the long-term follow up<sup>17</sup>. The incidence of consecutive XT tends to increase with time and multiple surgeries are strongly correlated with a high risk of consecutive XT<sup>18, 19</sup>. Correspondingly, multiple surgeries were also associated with the development of consecutive XT compared to the patients (without grouping) aligned with one surgery in our study ( $p = 0.002$ ). Twenty five percent of consecutive XT in early group seems to be high and this finding may be related with difficulty of the measurement of deviation at distance in the first year of life or long-term follow up of the present study. However, there was no statistically significant difference when compared to the other groups ( $p = 0.32$ ). The rate of consecutive XT did not vary with the level of VA, and amblyopia was not associated for the development of the consecutive XT in the present study ( $p = 0.76$ ).

The development of stereoaquity is correlated with several factors, including VA, ocular alignment, and intact cortical processing<sup>2</sup>. Stereopsis is not present at birth but starts to develop within the 3rd and 4th months of life and rapidly develops during the first 6 months of life in humans<sup>2, 20</sup>. Prospective studies showed that surgical alignment within the first 24 months of life is associated with better stereopsis<sup>10</sup>, especially within the period of plasticity at 2–4 months of age for the development of stereopsis<sup>14</sup>. In final report of the “early vs late infantile strabismus surgery study” (ELISSS), cases with early surgery (between 6–24 months) had better stereopsis in comparison with the cases with delayed surgery (between 32–60 months) at a 6-year follow up<sup>21</sup>. However Helveston et al.<sup>1</sup> reported that only 4 (25%) cases had stereopsis in their 10 cases of infantile ET who had BMR surgeries within 6 months (83–159 days) of age with a long-term follow up, and they pointed out that the development of stereopsis could be increased with early surgery and stereopsis might be developed by structural factors rather than the timing of the surgery. Although duration of misalignment within the critical period has been found to be the most important factor for the development of stereoaquity in some studies, the results of the present study show that early surgical alignment is not associated with better stereoaquity outcomes. No significantly greater proportion of children in the early surgery group developed stereopsis than children in the other groups. In our study even with early surgery, only 35% of the children developed gross stereopsis (Titmus-Fly) and no one developed high-grade stereopsis (40–60 seconds of

arc). Furthermore, all children (without grouping) with any degree of stereopsis have better VA ( $p = 0.03$ ).

DVD is the most common manifestation of the dissociated strabismus complex. Early alignment has not prevented development of dissociated strabismus<sup>1</sup>. The longer a patient is followed after alignment for congenital ET (up to 6 years of age), the more likely DVD is to be found<sup>22</sup>. In one study DVD developed in 34.8% of the cases who had undergone bimedial rectus recession<sup>23</sup>. Rate of DVD was 40% in the early group in the present study. When compared with the other groups this difference was statistically significant ( $p = 0.03$ ). DVD development age was also earlier in the group I ( $p = 0.03$ ). DVD is present in 35.3% of all patients (without grouping) who develop consecutive XT following surgical correction of infantile esotropia ( $p = 0.07$ ). In our study, the incidence of DVD in early group was similar to other studies. However, Arslan et al.<sup>23</sup> reported that it may be an underestimation in the other groups due to difficulties in differential diagnosis in patients with IOOA coexisting DVD. In contrast to Arslan et al.<sup>23</sup> study, early surgery made more likely to have DVD development in the present study.

IOOA is commonly associated with infantile ET. In a study, IOOA was reported in 48% of the patients with infantile ET<sup>24</sup>. However, Eustis et al.<sup>25</sup> reported that development of IOOA in infantile ET was 55%. In the present study, IOOA was developed in 50.9% of all cases by time and the difference between the rates of IOOA in the groups was insignificant ( $p = 0.62$ ). However, mean age of the patients for the development of IOOA was significantly lower in the early surgery group with respect to the other groups ( $p = 0.006$  and  $p < 0.001$ , respectively).

This study has some limitations. The sample size was small, the study was a non-randomized retrospective clinical trial that was from a single institution with a limited number of cases and the data provided may not be representative of other medical centers in our country. In this study we excluded cases with IOOA or DVD preoperatively to determine the development rate and time of these pathologies. However these two conditions are more difficult to identify in younger patients and also tend to present later after the infantile esotropia, regardless of surgical intervention. The excluding DVD and IOOA in the groups II and III might lead to introduce a selection bias. While difficult to confirm, one would assume that many of those children in the first group would have developed DVD and IOOA if they had waited on surgery.

### Conclusion

Patients with infantile ET have potential of gross stereopsis but high grade stereoacuity cannot be obtainable in the majority of them despite the early alignment of eyes. Not only the age of ocular alignment but also some constitutional factors, mentioned by Helveston et al.<sup>1</sup>, seem to have some effects on the development of binocular vision with high-grade stereo acuity in patients with infantile ET who are surgically treated. Early surgery also has potential effects for the development of both IOOA and DVD earlier. Early surgery should be performed to patients with infantile ET considering the advantages and disadvantages of this procedure. Very early surgery (< 6 months) may have better results but this study does not include such cases.

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## Increased accuracy of single photon emission computed tomography (SPECT) myocardial perfusion scintigraphy using iterative reconstruction of images

Povećana tačnost *single photon emission computed tomography* (SPECT) perfuzione scintigrafije miokarda korišćenjem iterativne rekonstrukcije

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### Abstract

**Background/Aim.** Filtered back projection (FBP) is a common way of processing myocardial perfusion imaging (MPI) studies. There are artifacts in FBP which can cause false-positive results. Iterative reconstruction (IR) is developed to reduce false positive findings in MPI studies. The aim of this study was to evaluate the difference in the number of false positive findings in MPI studies, between FBP and IR processing. **Methods.** We examined 107 patients with angina pectoris with MPI and coronary angiography (CAG), 77 man and 30 woman, aged 32–82. MPI studies were processed with FBP and with IR. Positive finding at MPI was visualization of the perfusion defect. Positive finding at CAG was stenosis of coronary artery. Perfusion defect at MPI without coronary artery stenosis at CAG was considered like false positive. The re-

sults were statistically analyzed with bivariate correlation, and with one sample *t*-test. **Results.** There were 20.6% normal, and 79.4% pathologic findings at FBP, 30.8% normal and 69.2% pathologic with IR and 37.4% normal and 62.6% pathologic at CAG. FBP produced 19 false-positive findings, at IR 11 false positive findings. The correlation between FBP and CAG was 0.658 ( $p < 0.01$ ) and between IR and CAG 0.784 ( $p < 0.01$ ). The number of false positive findings at MPI with IR was significantly lower than at FBP ( $p < 0.01$ ). **Conclusion.** Our study shows that IR processing MPI scintigraphy has less number of false positive findings, therefore it is our choice for processing MPI studies.

### Key words:

angina pectoris; diagnosis; scintigraphy; coronary angiography; technology, medical; methods.

### Apstrakt

**Uvod/Cilj.** Filtrovana projekcija unazad (FBP) uobičajen je način za obradu slika dobijenih pomoću SPECT perfuzionom scintigrafijom miokarda (MPI). FBP obradom moguća je pojava artefakata koji mogu da dovedu do pojave lažno pozitivnih rezultata. Iterativna rekonstrukcija (IR) razvijena je sa ciljem redukovanja lažno pozitivnih rezultata na MPI scintigrafijama. Cilj ove studije bio je procena razlike u broju lažno pozitivnih rezultata na MPI scintigrafijama, korišćenjem FBP i IR. **Metode.** Ukupno smo pregledali 107 bolesnika sa anginom pektoris, 77 muškaraca i 30 žena, starih od 32 do 82 godine, koristeći SPECT MPI scintigrafiju i koronarnu angiografiju (CAG). MPI scintigrafske slike su rekonstruisane korišćenjem FBP i IR. Pozitivan nalaz na MPI scintigrafijama bio je vizualizacija perfuzionog defekta. Pozitivan nalaz na CAG bio je vizualizacija stenozne koronarne arterije. Perfuzioni defekt na MPI bez stenozne

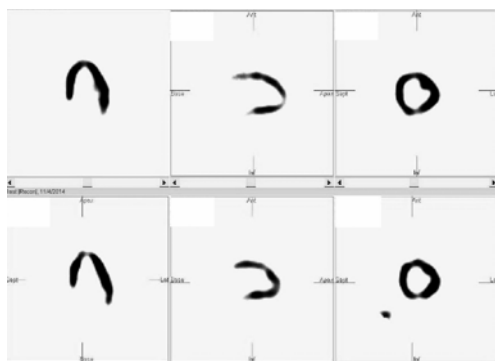
koronarne arterije na CAG smatran je lažno pozitivnim MPI rezultatom. Rezultati su statistički analizirani bivarijantnom korelacijom i *t*-testom. **Rezultati.** Na MPI scintigrafijama obrađenim postupkom FBP bilo je 20,6% normalnih, i 79,4% patoloških rezultata. Ukupno 30,8% normalnih i 69,2% patoloških rezultata dobili smo iz IR, dok je na CAG bilo 37,4% normalnih i 62,6% patoloških rezultata. Tehnika FBP je pokazala 19 lažno pozitivnih rezultata, dok smo pomoću IR dobili 11 lažno pozitivnih nalaza. Povezanost rezultata FBP i CAG iznosila je 0,658 ( $p < 0,01$ ), a povezanost IR i CAG 0,784 ( $p < 0,01$ ). Broj lažno pozitivnih rezultata bio je značajno niži primenom IR ( $p < 0,01$ ). **Zaključak.** Naš rad pokazuje da IR MPI scintigrafije ima manji broj lažno pozitivnih rezultata, zbog čega je to naš metod izbora za rekonstrukciju MPI studija.

### Ključne reči:

angina pektoris; dijagnoza; scintigrafija; angiografija koronarnih arterija; tehnologija, medicinska; metodi.

## Introduction

Filtered back projection (FBP) is the oldest and the most common way of processing single photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI) studies<sup>1</sup>. It is a rapid way to get images of the left ventricle with good contrast and respectable accuracy in assessing myocardial perfusion. But, there are possible arti-



**Fig. 1 – Patient 31: filtered back projection processed myocardial perfusion imaging with perfusion defects in the anterior wall and apex of the left ventricle.**

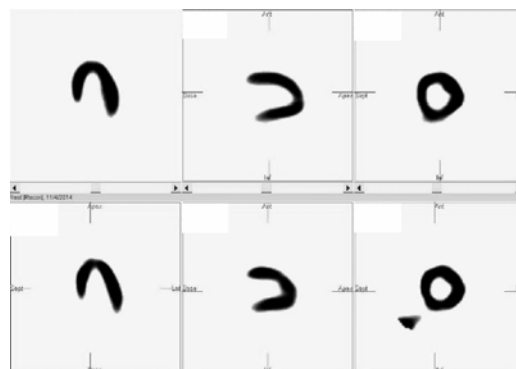
facts related to scattering of gamma photons within adjacent structures, which can cause the onset of the false positive results. Iterative reconstruction (IR) is developed with the aim to reduce the impact of attenuation and scatter, and in the final to avoid false positive findings at MPI studies<sup>2</sup>. Although IR is more demanding way of processing MPI studies, nowadays, computing power of SPECT devices allows the use of almost every processing modality<sup>3,4</sup>.

The aim of this study was to evaluate the difference in the number of false positive findings with MPI studies, between FBP and IR processing.

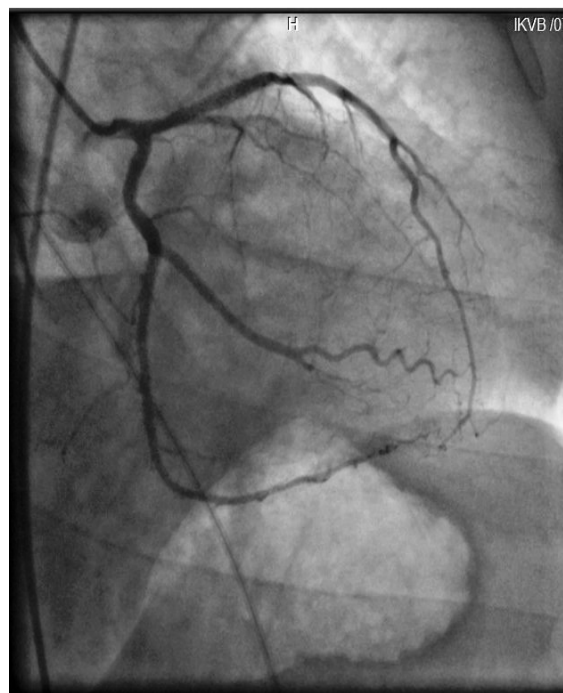
## Methods

We examined 107 patients with symptoms of angina pectoris. There were 77 men and 30 women, 32 to 82 years of age. For MPI studies we used  $^{99m}\text{Tc}$ -SESTAMIBI, standard two-day protocol, with exercise stress test<sup>5,6</sup>. All studies were done on a double headed E-Cam (Siemens) gamma camera, and processing was done on a processing station dedicated to it, with preinstalled INVIA software (® INVIA, LLC). MPI studies were FBP processed with order 5 and a cutoff of 0.7 and 0.5 Nyquist (0.53 cycles/cm)<sup>7-9</sup>, and after that we repeated reconstruction of MPI images with ordered subset expectation maximization (OSEM) IR. The number of iterations was 8 with 2 subsets. The images reconstructed with OSEM were filtered with a symmetric 3-D Gaussian function with full width at half maximum of 3.3 mm. All of the patients underwent CAG, with standard Seldinger approach. A positive finding for myocardial ischemia at MPI was visualization of the reversible perfusion defect in the myocardium of the left ventricle<sup>10</sup>. A positive finding at CAG was stenosis of coronary artery equal to or bigger than 50%. Perfusion defect

at MPI without coronary artery stenosis at CAG was considered as false positive MPI finding (Figures 1–3)<sup>11</sup>. The results of FBP, IR and CAG were statistically analyzed with bivariate correlation test and Pearson's correlation coefficient was calculated between the groups. Two tailed significance test was done, with the significance of correlation at the 0.01 level. The difference between false positive findings with FBP and IR was calculated with one sample *t*-test.



**Fig. 2 – Patient 31: iterative reconstruction processed myocardial perfusion imaging (normal perfusion).**



**Fig. 3 – Patient 31: coronary artery without stenosis on coronary angiography.**

## Results

There were 22 (20.6%) normal, and 85 (79.4%) pathologic findings at FBP, 33 (30.8%) normal and 74 (69.2%) pathologic with IR, and 40 (37.4%) normal and 67 (62.6%) pathologic findings at CAG. FBP produced 19 (17.8%) false positive findings, while with IR we got 11 (10.3%) false positive findings. The correlation between FBP and CAG was

0.658 ( $p < 0.00$ ), and between IR and CAG 0.784 ( $p < 0.00$ ). The number of false positive findings on MPI with IR was significantly lower than on FBP ( $p < 0.01$ ) (Table 1).

re time consuming for elder SPECT devices<sup>5</sup>. Computing power of newer devices makes this difference in the duration of computing insignificant. With IR processing it is possible

**Table 1**  
**Significantly smaller number of false positive findings at iterative reconstruction (IR) of myocardial perfusion imaging**

Technique	Patients (n)	Normal findings	Pathologic findings	False positive findings	Correlation with CAG
		n (%)	n (%)	n (%)	
FBP	107	22 (20.6)	85 (79.4)	19 (17.8)	0.658
IR	107	33 (30.8)	74 (69.2)	11 (10.3)	0.784
CAG	107	40 (37.4)	67 (62.6)		

FBP – filtered back projection; CAG – coronary angiography.

## Discussion

FBP processing MPI studies was a common way of processing. It is fast and reliable, but insufficient when there is the need for handling with scatter artifacts<sup>12</sup>. It was the method of choice, because less demanding in computing power, and the results were clinically acceptable<sup>13</sup>. However, scatter artifacts can cause the appearance of perfusion defects at MPI studies in the myocardium with normal perfusion *eg* appearance of false positive MPI finding<sup>14</sup>. IR processing for MPI scintigraphy is more demanding and mo-

re to avoid artifacts related to scatter or attenuation of gamma photons, which can result in perfusion defects at MPI, without existence of ischemia or stenosis of coronary arteries. Avoiding scatter artifacts which results in false positive finding, can increase diagnostic accuracy of MPI<sup>15</sup>.

## Conclusion

Our study shows that IR processing MPI scintigraphy has less number of false positive findings than processing with FBP, therefore it is our choice for processing MPI studies.

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## Carotid surgery today: an update after 14,000 carotid endarterectomy procedures

Karotidna hirurgija danas: novine nakon 14 000 karotidnih endarterektomija

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### Key words:

carotid arteries; carotid artery diseases; endarterectomy, carotid; vascular surgical procedures; angioplasty, balloon; anesthesia, local; platelet aggregation inhibitors; treatment outcome.

### Ključne reči:

aa. carotis; aa. carotis, bolesti; endarterektomija a. carotis; hirurgija, vaskularna, procedure; angioplastika, balonska; anestezija, lokalna; antiagregaciona sredstva; lečenje, ishod.

### Introduction

Atherosclerotic lesions of the extracranial part of carotid arteries are one of the most common causes of stroke. The relationship between carotid artery occlusive disease and neurologic function has been recognized for more than, 2000 years. According to Rufus of Ephesus (about 100 AD), the term carotid was derived from the Greek word *κάρως* (karios), meaning “to stun, to fall into deep sleep”. The reason for naming the artery was that compressing it causes the loss of consciousness – “deep sleep”<sup>1</sup>.

Hippocrates “apoplexy” in 400 BC was the first written trace of human attempt to portray conditions that we know today as transient ischemic attack (TIA) or reversible ischemic neurologic deficit<sup>1</sup>. Awareness of carotid arteries disease and surgical attempts to repair them led to the first successful carotid endarterectomy (CEA), performed by Michael DeBakey in 1953<sup>2</sup>.

Since then, carotid surgery has developed a lot, so today we have various techniques to detect and treat diseased carotid arteries<sup>1</sup>.

### Diagnostic improvement

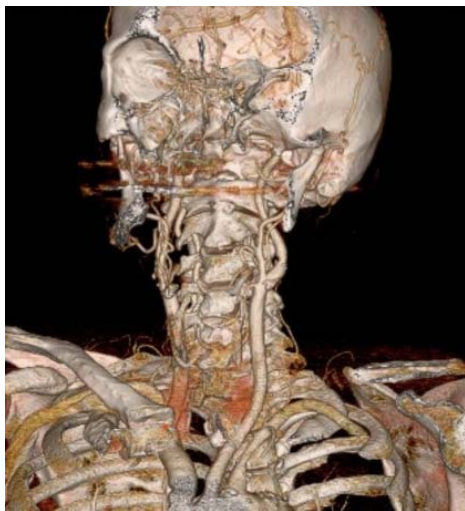
Preoperative imaging plays irreplaceable role in successful treatment of not just carotid arteries, but any organ, as well.

Carotid duplex scan (CDS) represents the first line in the diagnostics of carotid disease. This technology, which

combines the acquisition of anatomic and blood flow information, was developed in the 1970s<sup>3</sup>. Commercial duplex scanners became available by the 1980s, and the clinical use of CDS rapidly expanded in the past twenty years<sup>4</sup>. Modern CDS systems provided high-resolution B-mode ultrasound imaging of tissue and vessel anatomy, including 3D vessel reconstruction and evaluation of atherosclerotic plaque morphology, with detailed assessment of blood flow characteristics<sup>4</sup>.

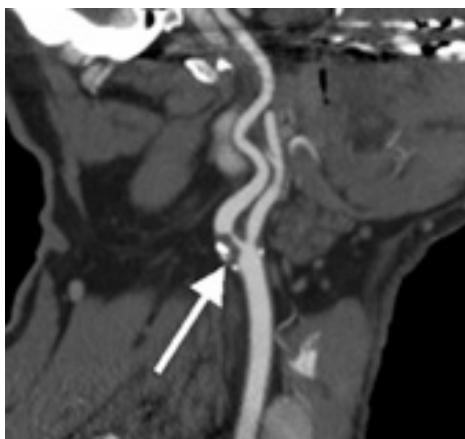
CDS is noninvasive and cost-effective and thus suitable for serial examination because it also reveals natural history of disease, including progression, regression, and response to intervention. In many patients, duplex testing can establish the definitive diagnosis the basis on which CEA or angioplasty can be performed<sup>4</sup>. However, the reliability of CDS depends on the expertise of the examiner and the interpreting physician.

When introduced in 1971, computed tomography (CT) scan brought a great improvement in the diagnostics<sup>5</sup>. Technological development led to the invention of 4-slice in 1998 and 16-slice multidetector CT (MDCT) scanner in 2002<sup>5,6</sup>. Later, the 256-slice MDCT provided ability to generate “real time” 3D images (Figure 1), for about the same amount of radiation as previous MDCT scanners<sup>5</sup>. Also, increasing use of MDCT angiography provided better visualization of the cerebral arteries, leading to an unexpected more frequent detection of unruptured intracranial aneurysms (UIAs)<sup>7</sup>.



**Fig. 1 – 3D model of the carotid arteries.**

Dual source MDCT scanners, developed in 2006, could precisely evaluate plaque distribution in the arteries, as well as they could make difference between calcified and lipid-rich plaques (Figure 2), which is of great importance in pre-operative planning<sup>5,8,9</sup>.



**Fig. 2 – Calcified and lipid rich component of atherosclerotic plaque.**

Because scanning with MDCT is done quickly, less contrast material can be administered at a faster rate, improving visibility of arteries<sup>5,6</sup>. Rapid patient throughput redu-

ces breath-holding time and received amount of radiation, which provides greater comfort for patients<sup>5,6</sup>. It also raises department productivity and saves money<sup>6</sup>.

Apart from the hardware type of a scanner, post-processing software and image management tools continue to evolve and warrants upgrade in a timely fashion.

The future of CT imaging lies in the fusion imaging technique. As a technology which enables synthesis of two dependent imaging modalities, it provides image with greater information. Positron emission tomography (PET)-CT fusion is the most relevant in the current clinical practice<sup>5</sup>.

### **Eversion carotid endarterectomy**

Although first described by DeBakey in 1959<sup>10</sup>, the modern technique of eversion CEA (ECEA) was introduced in the early 1970s<sup>11</sup>. However, its use became more popular about twenty years later<sup>12</sup>. At the end of the 20th and the beginning of the 21st century, surgeons worldwide began to report better outcomes following ECEA compared to the conventional technique (Table 1).

The majority of studies showed lower incidence of early postoperative death and neurological complications (seven days after surgery)<sup>1-14, 16-25</sup> in group treated with eversion technique. Also, incidence of late restenosis (follow-up period  $36.4 \pm 15.8$  months<sup>24</sup>) was much lower in patients treated with ECEA versus conventional CEA (Table 2).

In addition, Gao et al.<sup>28</sup> documented surprisingly lower incidence of postoperative microembolic events in ECEA, compared to the standard endarterectomy.

One of the world's largest single-center series of ECEA, by Radak et al.<sup>12</sup>, compared outcomes in patients operated on between 1991 and 1997 with those operated on in 1998–2004 period of time. The total mortality and morbidity rates and early postoperative complications were lower in the latter group. The clamping time was shorter, as well as was duration of hospital stay. At follow-up, rate of restenosis > 50% did not differ between the groups, but the incidence of < 50% restenosis was higher in the earlier group.

Improved surgical skills, shorter clamping time and better medication therapy led to preferable outcomes in patients operated on between 1998 and 2004<sup>12,25</sup>.

As a technique, ECEA offers lower restenosis rates and

**Table 1**  
**Early death rates following conventional and eversion carotid endarterectomy**

The author and the year	Patients (n)		Early death (%)	
	CCEA	ECEA	CCEA	ECEA
Darling et al. 1996 <sup>13</sup>	353	449	2.0	1.1
Entz et al. 1997 <sup>14</sup>	715	793	1.8	0.5
Cao et al. 1997 <sup>15</sup>	240	274	1.2	0.7
Cao et al. 1998 <sup>16</sup>	675	678	1.3	1.3
Shah et al. 1998 <sup>17</sup>	410	1,575	2.2	1.0
Ballotta et al. 1999 <sup>18</sup>	167	169	2.9	0.0
Peiper et al. 1999 <sup>19</sup>	388	475	1.5	1.1
Radak et al. 2000 <sup>20</sup>	682	2,124	1.3	0.5
Katras et al. 2001 <sup>21</sup>	204	118	1.3 + 2.8	0.8
Littooy et al. 2004 <sup>22</sup>	125	64	0.8	0.0
Marković et al. 2006 <sup>23</sup>	98	101	3.1	1.0

**CCEA – conventional carotid endarterectomy; ECEA – eversion carotid endarterectomy.**



**Table 2**  
**Long-term restenosis rates following carotid endarterectomy**

Author and year	Patients (n)		Late restenosis (%)	
	CCEA	ECEA	CCEA	ECEA
Cao et al. 1997 <sup>15</sup>	240	274	6.9	2.2
Cao et al. 1998 <sup>16</sup>	675	678	4.1	2.4
Shah et al. 1998 <sup>17</sup>	410	1,575	1.1	0.3
Ballotta et al. 1999 <sup>18</sup>	167	169	1.2	0.0
Peiper et al. 1999 <sup>19</sup>	388	475	10.2	2.5
Radak et al. 2000 <sup>20</sup>	682	2,124	1.8	0.5
Cao et al. 2000 <sup>26</sup>	/	/	9.2	3.6
Katras et al. 2001 <sup>21</sup>	204	118	6.5	1.7
Littooy et al. 2004 <sup>22</sup>	125	64	4.9	3.1
Ballotta et al. 2004 <sup>27</sup>	302	848	0.6	0.5
Marković et al. 2006 <sup>23</sup>	98	101	6.1	0.0

CCEA – conventional carotid endarterectomy; ECEA – eversion carotid endarterectomy.

greater technical ease of performance<sup>16, 25</sup>. Also, ECEA has been identified as an independent factor contributing to a better outcome following surgery<sup>29</sup>.

Still, there are certain relative contraindications to ECEA: restenosis after previous CEA, carotid stenosis due to radiation, ipsilateral surgical intervention in the past and lesions above second cervical vertebra<sup>24, 30–33</sup>. Therefore, alternative techniques have evolved.

### Carotid artery stenting

The endovascular era began, by seeking less invasive alternative to open surgery. First successful results of percutaneous transluminal angioplasty (PTA) of carotid arteries were reported by Matthias<sup>34</sup> in 1977 and Kerber et al.<sup>35</sup> in 1980. Balloon-expandable stents were first deployed in 1989, with improving of stent material and technique over years<sup>36</sup>.

Despite the early enthusiasm, the high likelihood of embolic stroke during carotid artery stenting (CAS) remained a major concern. The development of embolic protection devices (EPDs) in the 1990s has lowered the incidence of microembolization and consequent neurologic deficit<sup>24, 36–38</sup>.

From the beginning of the modern endovascular period, the procedure has been largely scrutinized. Four main inno-

ventions led to dissemination of CAS technique after 2000: routine use of stenting; routine use of EPDs; introduction of new stent materials for carotid endovascular procedures and new antiplatelet drugs<sup>36</sup>.

However, the question of CAS as an “equivalent” therapeutic option to CEA still remained. Five large randomized clinical trials were conducted seeking for answers to this dilemma (Table 3).

Except stent protected angioplasty vs carotid endarterectomy (SPACE) and international carotid stenting study (ICSS) (27% and 72%, respectively), in other trials EPDs were used in > 90% of cases. Asymptomatic patients were enrolled in carotid revascularization endarterectomy vs stenting trial (CREST) and stenting and angioplasty with protection in patients at high risk for endarterectomy (SAPPHIRE) trial.

The results implied to a higher perioperative stroke risk with CAS compared with CEA when it is performed in unselected patients with symptomatic or asymptomatic carotid stenosis. The association between older age and increased risk of adverse events after CAS, was reported in CREST, ICSS and SPACE trial<sup>43–47</sup>. The low absolute risk of recurrent stroke in CREST suggests that both CAS and CEA are clinically durable<sup>47</sup>.

**Table 3**  
**Trials investigating outcomes of carotid artery stenting (CAS) compared to carotid endarterectomy (CEA)**

Trial and year	n	Primary endpoint	Follow-up period	Results (%)	
				CAS	CEA
SAPPHIRE <sup>39, 40</sup> 2004, 2008	334	30-day stroke, MI, death + 1-y ipsilateral stroke, death	1-y	12.2	20.1
EVA-3S <sup>41, 42</sup> 2006, 2008	527	30-day stroke, death	30-day	9.6	3.9
SPACE <sup>43–45</sup> 2006, 2008	1,196	4-y ipsilateral stroke + death	4-y	11.1	6.2
SPACE <sup>43–45</sup> 2006, 2008	1,196	30-day ipsilateral stroke, death	30-day	6.9	6.5
SPACE <sup>43–45</sup> 2006, 2008	1,196	2-y ipsilateral stroke + death	2-y	9.5	8.8
ICSS <sup>46</sup> 2010	1,713	120-day stroke, MI, death	120-day	8.5	5.2
CREST <sup>47</sup> 2010	2,502	30-day stroke, MI, death	30-day	5.2	4.5
CREST <sup>47</sup> 2010	2,502	4-y ipsilateral stroke	4-y	7.2	6.8

CREST – carotid revascularization endarterectomy vs stenting trial; EVA-3S – endarterectomy vs angioplasty in patients with symptomatic severe carotid stenosis; ICSS – international carotid stenting study; MI – myocardial infarction, n – number of patients; SAPPHIRE – stenting and angioplasty with protection in patients at high risk for endarterectomy; SPACE – stent-protected angioplasty vs carotid endarterectomy; y – year.

The risk of periprocedural myocardial infarction after CAS was reported with rates about half as those of CEA: 1.1% vs 2.3% in CREST, 0.4% vs 0.6% in ICSS and 0.4% vs 0.8% in endarterectomy vs angioplasty in patients with symptomatic carotid stenosis (EVA-3S) trial<sup>41,46,47</sup>.

CAS should not be considered as an therapeutic option in patients with severe peripheral artery stenosis, aortic arch anomalies, carotid artery kinking/coiling and aneurysm, pre-occlusive lesions of the internal carotid artery (ICA), carotid stenosis longer than 2 cm, calcified, ulcerated or highly vulnerable carotid plaque and chronic renal insufficiency<sup>24</sup>. In such cases, open surgery remains the gold standard.

Currently, data suggest that with a careful patient and operator selection and improved technology, CAS may be considered as an alternative to CEA<sup>36,39-47</sup>.

### Hybrid procedures

Significant atherosclerotic lesions involving carotid bifurcation and the proximal ipsilateral common carotid artery (CCA) or the innominate artery (IA) are uncommon, with the reported incidence of approximately 4.8%<sup>48</sup>. However, their treatment remains a great challenge. Standard CEA exposure does not allow repair of the proximal IA or CCA; it could be approached through a median sternotomy, occasionally requiring cardiopulmonary bypass<sup>49</sup>. On the other side, the access to skull base-level ICA stenosis mandates mandibular subluxation<sup>50</sup>. These procedures are associated with a prolonged operative time, increased blood loss and increased morbidity/mortality incidence<sup>51</sup>.

In 1996, Diethrich et al.<sup>52</sup> described a new, hybrid technique for simultaneous treatment of carotid bifurcation and proximal lesions. This procedure consisted of surgical exposure of carotid bifurcation, retrograde stenting of the proximal CCA or IA lesions, followed by CEA.

A meta-analysis by Sfyroeras et al.<sup>53</sup> reported a 30-day periprocedural stroke and mortality rates of 1.5% and 0.7%, respectively. During follow-up, the incidence of restenosis in patients treated with stenting was 3.7% vs 14% in patients that received simple balloon angioplasty, further signaling that proximal lesions should be solved with stent implantation. In order to assure better outcomes in patients that did not receive antiplatelet therapy, an increased dose of clopidogrel (450 mg) should be delivered immediately before the intervention<sup>54</sup>. Comparing mortality rates of hybrid procedures and open surgical approach (0.7% vs 0.5%–18.7%), stands clear that the hybrid technique made significant breakthrough in treatment of simultaneous lesions<sup>53</sup>.

The results of hybrid procedures label that CEA and CAS should not be considered as competitive, but complementary techniques.

### Local anesthesia

CEA can be performed using general anesthesia (GA) and/or local anesthesia (LA). LA comprises deep and superficial cervical block. Cervical block anesthesia (CBA) has evolved over the last 15 years with new techniques, novel methods of locating the cervical plexus and new drugs<sup>55-57</sup>.

Using CBA, neurological function is easily assessed during carotid crossclamping, with predictable haemodynamic control. In patients with significant cardiopulmonary comorbidities or in which GA is contraindicated, LA represents a safe and effective option. Disadvantages of LA include risk of seizure or allergic reaction, discomfort for some patients and anxiety for the operating surgeon<sup>58</sup>.

The general anaesthesia vs local anesthesia for carotid surgery (GALA) trial was designed to compare outcomes in patients operated on under GA or LA. The results showed no significant difference in the incidence of stroke, myocardial infarction or death at a 30-day follow up. Adverse cardiovascular events were reported in 4.8% of patients who underwent CEA under GA and 4.5% of patients who underwent CEA under LA. Also, there was no difference in hospital length of stay between the groups<sup>59</sup>.

Since the GALA trial, several other studies have also reported subtle differences between GA and LA<sup>55</sup>. Referring to the results of these reports, we can say that the efficacy of vascular team looking after the patient is more important than the choice of anesthetic technique itself. Since no data showed predominance of GA or LA, selection of suitable anesthetic method remains to be discussed between the patient, his surgeon and anesthesiologist.

### Best medical treatment

The invention of new, potent drugs, led to an idea of creating a novel therapeutic modality for carotid arteries disease, named the best medical treatment (BMT).

One of the most significant improvements has been the aggressive use of antiplatelet therapy, and early studies reported up to 25% reduction in overall stroke rates among patients undergoing CEA<sup>58</sup>.

When clopidogrel bisulfate was approved by the Food and Drug Administration (FDA) in 1998<sup>60</sup>, a new chapter in antiplatelet therapy has been opened.

Addition of a single 75 mg dose of clopidogrel to a regular 75 mg dose of acetylsalicylic acid (ASA), administered the night before CEA, was associated with a significant reduction in postoperative neurological events, without any increase in hemorrhagic complications<sup>61</sup>. A study of Sharpe et al.<sup>62</sup> showed a lower incidence of postoperative microembolisation in patients receiving dual therapy, compared to patients receiving only ASA. Also, the need of adjuvant dextran therapy was reduced.

The utility of dual antiplatelet therapy use after CAS has been observed in the management of atherothrombosis with clopidogrel in high risk patients (MATCH) with recent transient ischemic attack or ischemic stroke and the clopidogrel for high atherothrombotic risk and ischemic stabilization, management and avoidance (CHARISMA) trials<sup>63-65</sup>. The benefit of combination therapy was found to be significant in patients with symptomatic carotid disease; conversely, the related risk of bleeding obviated the benefits of treatment in patients with low risk of postprocedural neurologic complications<sup>64,65</sup>. A recent study showed that the use of ASA and clopidogrel 4–6 weeks after CAS is suffi-

ent to decrease the risk of ischemic stroke, composite vascular events or death<sup>66</sup>.

Apart from antiplatelet agents, lipid lowering drugs have significant role in prevention of major cardiovascular events. Since lovastatin had been commercialized in September 1987, 6 statins, including 2 semi-synthetic statins (simvastatin, pravastatin) and 4 synthetic statins (fluvastatin, atorvastatin, rosuvastatin, pitavastatin) have been introduced<sup>67-69</sup>. Statins have both lipid-lowering and anti-inflammatory effects, and have been shown to reduce risk of neurologic events in symptomatic patients and in patients after CEA or CAS<sup>70-72</sup>. Furthermore, it appears that the stroke prevention benefits of statins are related to their pleiotropic effects rather than their cholesterol lowering effects<sup>58</sup>.

Numerous trials and meta-analyses since the mid-1990s, revealed a strong correlation of statin use and reduced stroke risk<sup>58, 73-75</sup>. In a recent series of 1,566 patients who underwent CEA, at a 30-day follow-up, statins were found to be associated with a reduced incidence of death (0.3% vs

Recently, there has been an increasing argument to favor BMT as stand-alone treatment in all neurologically asymptomatic individuals, regardless of the degree of carotid stenosis<sup>78</sup>. This argument deserved serious critical analysis, because the majority of carotid interventions currently performed are in asymptomatic patients. In an effort to address the question of BMT in patients with confirmed carotid disease, Abbott et al.<sup>79</sup> performed meta-analyses of 11 trials that included 3724 patients with  $\geq 50\%$  carotid stenosis. Comparing their results with the results from endarterectomy for asymptomatic carotid artery stenosis (ACAS) trial, they determined that the contemporary risk of ipsilateral stroke and/or TIA did not differ, and possibly were better than the results reported for ACAS in 1995.

The latest data, which provides the most contemporary comparison of BMT alone to BMT plus CEA or CAS, indicate that CEA followed by medical therapy represents the best modality in reduction of cardiovascular events.

**Table 4**  
**Effect of best medical therapy and risk factors reduction on major adverse cardiovascular events (MACEs)**

Treatment	Comment	Outcome
Antiplatelet therapy	Single or dual therapy	Reduces both stroke rate and overall MACEs
Antihypertensive therapy	Decrease BP by 10 mm Hg systolic/5 mm Hg diastolic or to 120/80 mm Hg. Treat all patients regardless of baseline BP.	Reduces stroke recurrence and restenosis rates
Lipid lowering therapy	Reduce LDL by 50% or < 70 mg/dL. Treat hyperlipidemia and normolipemic patients with history of stroke.	May be beneficial if applied prior to CEA/CAS
Smoking cessation	Total abstinence	Reduces stroke and MACEs rates
Alcohol consumption	Avoid excessive consumption	Reduces overall MACEs rates

**BP – blood pressure; CAS – carotid artery stenting; CEA – carotid endarterectomy; LDL – low density lipoprotein.**

2.1%), stroke (1.2% vs 4.5%) and TIA (1.5% vs 3.6%). A fivefold lower risk of death and a threefold lower risk of stroke was found in statin users group<sup>76</sup>. In a single-center experience, the incidence of cardiovascular events after CAS was 4% in statin users vs 15% in nonusers<sup>77</sup>. Another large series showed 1.5% vs 4% 30-day stroke/death rates for users and non-users<sup>72</sup>.

In addition, according to multi-center experience, dual antiplatelet therapy and statins with angiotensin-converting enzyme (ACE) inhibitor or beta blocker after CEA or CAS, resulted in a lower incidence of restenosis and adverse postoperative effects (Table 4)<sup>12, 36, 58, 76, 78</sup>.

## Conclusion

Overviewing the last 20 years, we stepped forward in understanding, diagnosing and treating carotid arteries disease. More sophisticated preoperative imaging, improved surgical skills, development of new stent materials and techniques and new medication therapy led to better outcome following carotid arteries treatment. Yet, there is a long way to go in order to reduce incidence of peri- and postoperative adverse effects, especially in high risk patients and the elderly population. The saga continues...

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## Multichannel learning for training medical staff in Serbian Army Forces

Višekanalno učenje za obuku medicinskog osoblja u Vojsci Srbije

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**Key words:**  
education, distance; medical staff; military personnel; serbia.

**Ključne reči:**  
obrazovanje na daljinu; kadar, medicinski; kadar, vojni; srbija.

### Introduction

The implementation of distributed e-learning in the Serbian Army Forces (SAF) formally started in the beginning of 2010 and was realized through the two biannual projects (2010–2011 and 2012–2013) in cooperation with the Norway Ministry of Defense and the Jefferson Institute. As a result, two Web applications were developed: a distance learning info-portal (<http://elearning.mod.gov.rs/>) and a distance learning platform (<http://adl.elearning.mod.gov.rs/>). Military medical staff recognized the importance of such a system to meet their requirements during frequent missions abroad as part of UN and EU forces. At any moment, there are dozens of SAF nurses, medical technicians, specialists and surgeons on duty in every part of the world and they need access to E&T resources. Therefore, the first e-courses were built for their demands in order to provide efficient and effective delivery of E&T resources remotely, to provide contextualized communication and to make useful reminders available for personnel engaged in missions abroad where E&T resources have great utility<sup>1</sup>.

Missions abroad are often in undeveloped countries with a poor Internet infrastructure especially in rural areas everywhere. It represents one of difficulties which continuously motivate us to make some improvements. Multichannel learning<sup>2</sup>, described in this paper, was recognized as an appropriate approach: delivering the same resources in an appropriate form and manner on any platform – desktop or laptop computer as well as tablet or smart-phone. In this

way, a user can access the learning content through either the regular Internet connection, or a mobile network. The description and preliminary results of implementing multichannel learning are represented here.

### Motivation and solution

One of the SAF missions is to participate in building and maintaining peace in the region and across the world. The Serbian Army has been significantly engaged in missions abroad since 1956 (14,265 persons have been engaged in 22 rotations). The personnel are working in very difficult conditions, far away from home, in improvised or field facilities adapted for performing mission tasks. There have been many situations in which they must make a decision in minutes, or even worse – in seconds without any kind of expert support. Consultations through telephone calls are too expensive. The resources offered at deployment location that are usually brought along by them often cannot meet all their needs. The solution for such a situation was recognized in distribution of E&T resources to the distant locations by contemporary Internet technologies. The SAF distance learning platform as a robust and secure e-learning platform provides E&T content, communication and collaboration features to users regardless of distance and roles (instructors or trainees)<sup>3</sup> that motivated us for development of several medical courses. These are first aid courses, human immunodeficiency virus (HIV) protection courses and an Academy of Maritime Education and Training University (AMET) course. Some of

them are the basic level courses accessible to every registered user. Once released, these courses proved to be very useful, as measured by to the number of users and the cumulative time spent in using their resources. Every course has hybrid structure (hierarchical and sequential). More complex content has a hierarchical structure on the upper levels while the concrete materials within one learning unit can be sequentially ordered. Its content consists of illustrated text pages, video clips and question pages for self testing.

The user can adapt the E&T pace according to his plans and time. He can also contact other persons that attend the same course by using instant messaging offered by the platform (Figure 1). In such a scenario, the user can make himself visible or not for others. For instance, if a concrete user needs help or offers help to others he/she should be visible. If he/she wants to be undisturbed during the learning time, he/she just makes him/her self hidden.

Topic forums and blogs represent another way for communicating with others. It is very useful if the user looks for specific information but does not know the appropriate person(s) that could help him/her. This is the so-called collaborative learning approach, a part of social constructivism theory<sup>4</sup>.

### Design and implementation

In rural and sparsely populated areas the 3G mobile telephony network represents the only high quality Internet

connection available for multimedia and high speed communication support. That means that smart phones represent the only solution in this case. Application is available to every registered user on the e-learning platform<sup>5</sup>.

After logging, the system delivers the list of available courses to the user. After downloading the course, if selected mobile application starts the course. The mobile course is presented in the figure below (Figure 2). One of the main principles followed in the mobile courses design is to follow the same navigation principles as in their desktop versions. In this way their exploration is easier for trainees who previously used the desktop versions. Another important design principle is to keep navigation easy throughout the content. For clarity of appearance, text descriptions and explanations are removed. Pictures are used instead. The content structure is adapted to represent a clear hierarchy of learning chunks.

Some contents are represented only by short video clips to provide the users with a quick reminder to prepare themselves immediately before executing the tasks in stressful conditions. Theoretically, considering the circumstances in which mobile learning is expected to be used, learning is regarded as an emotional process in which the content's appearance, environmental and time constraints strongly influence the perceptive abilities of the learner<sup>6</sup>. Based on Dales cone of experience<sup>7</sup> in which it is explained that people generally remember 10% of what

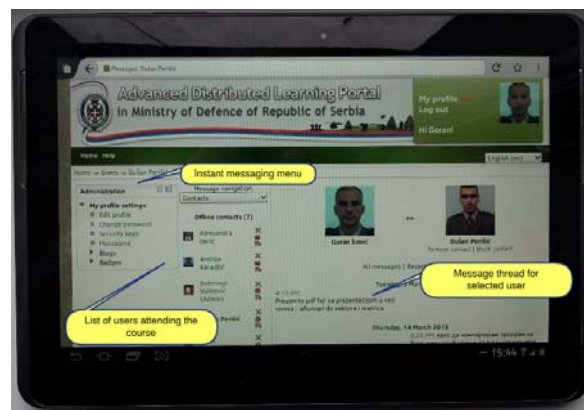


Fig. 1 – Serbian Army Forces (SAF) distance learning platform on the tablet – Instant messenger.



Fig. 2 – Keeps navigation easy through the course content.



they read, 20% of what they hear, 30% of what they see and 50% of what they hear and see at the same time, the content is redesigned to be comprised as much as possible of pictures and video materials.

Where it is possible, in order to keep the content simple and short, check lists are used instead of long text descriptions. This distilled form is especially useful as a reminder for strictly defined procedure (standard form for acting in emergency situation). When it is necessary to better explain particular steps of a certain procedure and to keep the description as simple as possible – an animated sequence of frames is well suited to the smart phone. In the next example (Figure 3), this tactic is used for describing the approaching the scene of an accident procedure. Together with the visual representation is an audio explanation for each bullet of the check list. Frames are time synchronized with the multimedia content that results in the cartoon effect. This is one way in which complex descriptions can be realized within the very limited resources of a mobile platform.

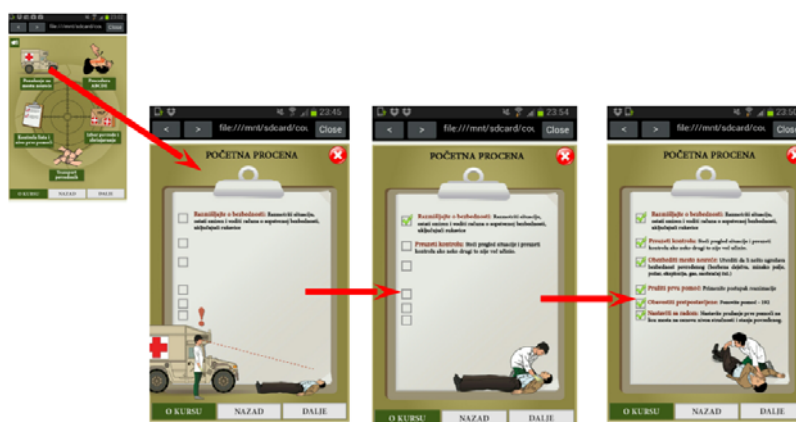
In this section, we presented the most important didactics applied in the mobile courses: simplified navigation, intensive usage of short video clips, making use of check lists as a form of distilled knowledge of procedures, and animated sequence of frames for presenting more complex content. There are other techniques that implement different pedagogical tactics for animating the users to combine the regular courses with mobile ones and to use advanced smart phone abilities. For instance, preparing themselves for assessments, arranging appointments and communicating with instructors,

and to find peers in neighborhood for help. These are beyond the scope of this paper and they are not presented in the text.

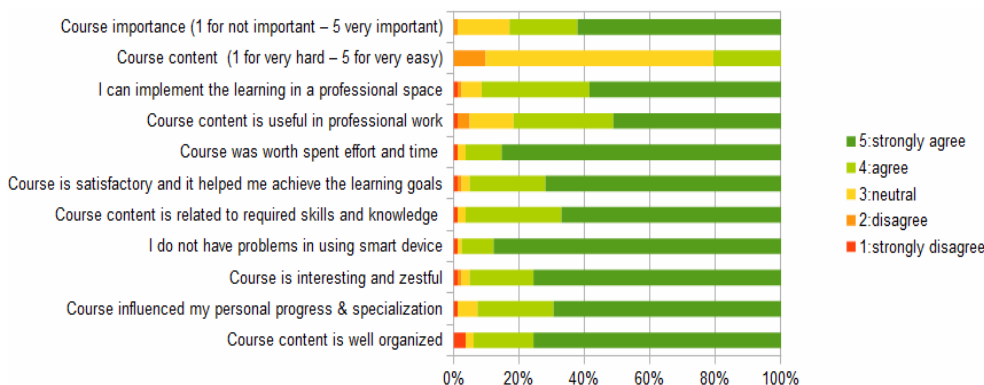
**Evaluation of the distance learning quality**

As mentioned, courses are designed for various types of users. Apart from medicals, more than a hundred other SAF members have been using the desktop and mobile courses since the middle of 2013. For evaluation of each course, a survey form was applied based on the Course Experience Questionnaire (CEQ) methodology<sup>8</sup>, which has been used at universities in Australia for more than seven years to measure the quality of teaching and learning. Following this approach, we developed a survey with 24 questions in the form of single choice answers with the possibility to add comments, and questions with 5-level Likert scales<sup>9</sup>. The single choice type is used for questions about general information. There were 82 persons who participated in the survey. The single choice questions yielded the following facts: almost 94% of respondents had no problems to access the courses; only 5 persons out of 82 encountered access challenges; approximately 60% of respondents used courses from home, while the rest were uniformly distributed – they used courses from the workplace, during a mission abroad, or on the terrain (open field missions); wireless medium was the predominantly used internet access – in more than 80% of cases, a 3G mobile network was used in others.

Every question has a default answer. If it is selected, the user is requested to answer the conditional question to



**Fig. 3 – Animated sequence of frames.**



**Fig. 4 – Evaluation of First Aid for Soldiers mobile course.**

explain his choice. Thus, we collected detailed information about environmental context in which the course resources are used. For instance, for those who answered "yes" to the first question (the user had a problem to access the course), 4 of 5 respondents mentioned lack of Internet access as a problem to use the courses.

Five-level Likert scaled questions are used for evaluating the course as a whole. The next illustration (Figure 4) shows the questions used and the user's answers. Predominant green areas show that the specified course is motivating, useful in professional work, interesting and zestful. Moreover, the users did not have problems in using smart devices. They also verified that the course content was not trivial (the 2nd question) and that it was well organized. Owing to the achieved results of survey, it is obvious that adapting medical courses and delivering them through the mobile channel is found very useful by potential users.

In the same way every particular topic in the course is evaluated with the purpose of this part of survey with the aim to collect more detailed information about quality of topical content. The questions are paired – one for clarity of the topic and the other for a topics' weight.

The subsequent conclusions follow: there is no answer pointing to strong disagreement; all of the topics are well explained; topics that consist of check lists are tackled much more easily than those without them. The survey also shows that paired questions about topics (clarity and weight) are in strong correlation and this is a welcome response because it means that weightier topics are better explained.

## Conclusion

The evaluation results led us to the conclusion that the persons participating in the survey are well skilled in using smart devices and they mostly do not have any problems to access courses during the evaluation period. Check lists should be used as much as possible and in combination with other types of content. For instance, the topics ABCD procedures, Injury detection and first aid care, and Transportation of an injured person are appropriate for this change.

Likert scaled survey designed for the course evaluation and evaluation of each topic brought successful results. Regardless of the heterogeneous structure of 82 users (13.4% female, 23% higher education, medical personnel together with others from different branches and services) the results give us insight and motivation for future development. Moreover, the users think that the enhanced course accessibility (deliverable anywhere at any time) could increase their self-confidence on the terrain as both versions (desktop and mobile) are deliverable.

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## Chernobyl and Fukushima nuclear accidents: What have we learned and what have we done?

Nuklearni akcidenti u Černobilju i Fukušimi: šta smo naučili, a šta učinili?

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### Key words:

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### Ključne reči:

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### Introduction

Construction of new blocks of the Nuclear Power Plant (NPP) PAKS 2 in Hungary last few months caused vigorous debate in the media. Most of the titles, as always when it comes to radiation, is sensationalistic and encourages fear and concern of the Serbian population because of the proximity of a nuclear installations to our border. As the professional community does not sound a lot, spreading of fear is undisturbed.

The exploitation of nuclear energy and the accelerated construction of NPPs began with the assertion that this is, unlike thermal power plants, environmentally clean, safe and secure energy production. However, reality shows that every ten years at least one large-scale nuclear accident happens, called major nuclear accident, which can endanger people and the environment. Currently there are over 440 NPPs operating in the world. None of them is in Serbia <sup>1</sup>.

This spring marks the 30th anniversary of the accident in the Chernobyl NPP, one of the largest in the history of the application of nuclear energy and 5 years from the accident at the Fukushima Daiichi NPP, which was just by chance not overcome by the harmful effects. Although geographically distant, these events had a profound influence on Serbia and entire European continent, not only by harmful effects on the health of the population and the environment, but also influenced public opinion and attitude towards nuclear energy as well as the defence and response organization in the event of accident. It became

definitely clear that accidents of this type, which are not limited by political and national borders, can only be tackled through joint and synchronized activities. This attitude has led to the launch of a number of joint activities under the auspices of international organizations (United nation – UN, the World Health Organization WHO, International Atomic Energy Agency – IAEA): education, synchronization, joint team creation, programs of mutual assistance and notification, mutual long-term monitoring of late effects.

Each of these accidents was analyzed in all respects to observe the slightest flaw and find solutions to overcome the observed and the prevention of recurrence of error. In most countries such accident was the cause to review their response plans in case of accident, and improve them by incorporating new knowledge. As members of the Team for response in the event of radiation accidents and consequence monitoring, we wanted to remind of both Chernobyl and Fukushima Daiichi accident.

The aim is to analyze preparedness of our country for the event of a new nuclear accident and whether we have reason for concern about the construction of new reactors in the NPP Paks 2 in Hungary.

### Chernobyl vs Fukushima Daiichi accident

It was written a lot about Chernobyl accident in previous years, so we will look back at it only through important

facts and for the purpose of comparison with other accident. Chernobyl accident happened on April 26th, 1986 as a consequence of two subsequent explosions in one of four nuclear reactors of the Chernobyl NPP (Unit 4). Explosions were caused by a few factors, but mostly by manmade mistakes and some technical imperfections. They caused large radioactive release in a very short time. Additionally, high temperatures in the reactor caused further melting of the remaining fuel, which caused prolonged radioactive emission for the next thirty days. It is estimated that the total amount of radioactive release was 5,300 PBq, 200 hundred times higher than radioactivity used on Hiroshima<sup>2,3</sup>. Radioactive cloud released at the time of explosion (450 PBq) was expelled 9 km high in the atmosphere, and later on carried by the winds over many European countries, first over Scandinavian countries, then changing direction of the air currents contaminated Poland, Czechoslovakia, southern parts of Germany, Austria, and finally South and Southeast winds accompanied by rain showers have led to contamination of Balkan countries. Most of the radioactive fallout was dispersed over Belarus, Ukraine and Russia<sup>4,5</sup>.

During the first three days of the accident iodine-131 (I-131) was the most dangerous for the health of residents of contaminated areas, and then dominated cesium-137 (Cs-137) and strontium-90 (Sr-90), and to a lesser extent, plutonium-241 (Pu-241)<sup>6</sup>. In addition to these radioactive elements, "hot particles" were found in the cloud, which had the same composition as irradiated nuclear fuel from the damaged reactor. From the very beginning Chernobyl accident was proclaimed as Major accident, according to the International Nuclear Events Scale (INES) level 7 event, which is the largest-scale accident, which endangers the environment and population. Employees of the NPP, as well as rescue teams, mainly firefighters, soldiers and medical teams with superhuman efforts and numerous human sacrifices did everything to prevent the worst. Estimated by expert commissions, Chernobyl accident, regardless numerous causes, was primarily the result of human error. The accident of such large scale was not even considered in the document for the risk assessment and plans for the Chernobyl NPP emergencies<sup>6</sup>.

On the contrary, Fukushima Daiichi accident did not arise suddenly, but developed over several weeks. This is the accident with highest number of human casualties and destruction and largest in Japan since World War II. At the same time it is a unique blend of natural disasters<sup>7,8</sup>. Problems on NPP began after the devastating earthquake of magnitude 9 at the moment magnitude scale and tsunami which followed very quickly. Although Japan is a country where earthquakes are not uncommon, this earthquake, whose epicenter was only 180 km from the NPP, for its strength, destructive power and the extent of the territory that was affected, was the fourth measured in the last 60 years. In the affected territory, with 5 NPP, with a total of 15 reactors, of which 11 were in operation, only in the NPP Fukushima Daiichi damage was caused to vital parts, while in the other NPPs damage was caused to the parts of the equipment which were of minor importance<sup>9</sup>.

In the NPP Fukushima Daiichi occurred severe core damage in three of the six reactors for which it was announced as the accident INES Level 3 event. At that time (March 12th) most of released materials were radioactive noble gases (xenon-133). At the time of the earthquake there were 6,400 employees in the plant and three days later, after the evacuation and willful abandonment of the NPP, remained about 700. At that time release of hydrogen and radioactive materials occurred and accident announced as INES level 5 event. As a result of earthquake, power supply from external sources was interrupted that caused stoppage of cooling system of the three operational reactors. Generators for emergency situations were later activated, but due to increase of temperature and pressure there were an explosion of released hydrogen and release of radioactive I-131 at the speed of  $10^{15}$  Bq/h and physical damage of three reactor buildings. At that time, INES level 6 event was announced. A fortunate circumstance was that the winds carried the radioactive cloud until March 15 towards the Pacific Ocean. On March 15 wind direction changed and the radioactivity was spilled on land north-west of the NPP, when accident was announced as level 7 INES scale event. Workers of NPP did not succeed in checking the extent of damage to the reactor and generator caused by earthquake, when tsunami came. The main tsunami was 13 m high. After it there was a flood, and a significant part of the plant found itself under water, at some places more than 5 m. That approach was unusable for repairs<sup>1</sup>.

The greatest part of radioactivity in the Pacific Ocean was spilled during March and April 2011 through contaminated water that flowed from the NPP and contained radioactive I-131 and Cs-137. That process, to a lesser extent, continued 5 years after the accident<sup>10,11</sup>. Although three reactors were involved in this accident, the estimated released radioactivity was 520 PBq, or 10% of radioactivity released from the Chernobyl accident. The greatest part of radioactivity was deposited in the Pacific Ocean during March and April that year<sup>3,11</sup>.

Japanese NPP in its Risk Assessment Act had instructions on the procedure in case of earthquake, tsunami or floods, but the intensity of each of the resulting disasters significantly exceeded the maximum assumed value made at the time of its construction. That act has been repeatedly corrected according to the recommendations of meteorological services, and although protective systems have been upgraded in accordance with the recommendations, it turned out that they were not sufficient to defend against each of the disasters occurring individually. The situation in which a NPP is simultaneously affected by three natural disasters was not even presumed in these documents. The defense was further aggravated by disruption of communication and reduction of the number of employees in terms of the increased need<sup>8</sup>. The workers who remained, bearing in mind that many of them received doses that significantly exceeded the permitted levels, showed a remarkable level of consciousness and even sacrificed their own lives. However, in this event a huge number of serious flaws was noticed, misjudgements and decisions, and above all a high degree of improvisation, since there was no plan to respond to that kind of accident. The-

refore, the conclusion of the commission that investigated the accident was that it was “manmade disaster – that could and should have been foreseen and prevented”. And that was despite the fact that the accident was initiated by disasters<sup>12</sup>.

These two accidents have confirmed that each nuclear accident is unique. According to the mechanism different, yet they have a lot of similarities. First among them is that, regardless of the high technology and the number of measures of control and protection, they can never assume the entire unwanted and risky situations. Second, that in such a contingency, regardless of the expertise of the staff and their extraordinary efforts, human error easily occurs.

#### *The consequences of accidents on environment*

In both accidents there was a significant irreversible contamination of environment. In case of the Chernobyl accident 150,000 km<sup>2</sup> of the territory of Russia, Belarus and Ukraine were highly contaminated, and therefore evacuated in the radius of 30 km. In the highly contaminated territories of Ukraine and Belarus at the time of the accident lived more than 8 million people, of whom 2 million children. Around 350,000 of them were evacuated or fled their homes. These zones are still largely uninhabited<sup>13,14</sup>.

European continent is less contaminated, but 45,000 km<sup>2</sup> of European countries, including Serbia, have high levels of contamination<sup>5</sup>. Never before in the history of the use of nuclear energy was one territory as contaminated. Taking into account that in addition to I-131 (half-life of 8 days), also present are Cs-137 (half-life 30 years) and Sr-90 (half-life 28 years), contamination of the territory can be considered long-term. According to the experts of Greenpeace, because of the high contamination of long-lived transuranic radionuclides, a radius of 10 km around the NPP will not be ready for the return of residents for tens of thousands of years.

People who live in the contaminated terrain are confronted with everyday problems of contaminated food and water. Cs and Sr enter the food chain mimicking their physiological analogues potassium and calcium thus contributing to the overall contamination of the living world. Over the past three decades, residents of border regions which are not displaced have limited the intake of radioactive substances in different ways, limiting dose, using foods that are not grown on contaminated territory, regime of diet in accordance with the level of contamination of certain foods (ETHOS project), etc. Today, 30 years later, there are no social benefits as in former years. People are forced to feed on agricultural products grown in the contaminated territories and feel abandoned and sacrificed from the society<sup>1,15</sup>.

Although the values of Cs and Sr in the environment have been reduced by half, there are areas where they are still high, such as forests and humid parts of the wetland (over 30% of the territory). Forests are a kind of reservoir of contamination because they cannot be decontaminated and can scatter contamination in different ways. Inhabitants of these regions use natural products (mushrooms, forest fruits blueberries, venison and fish) daily, significantly raising the

ir radioactivity intake. Cs-137 level of mushrooms samples is 16 times higher than allowed. Samples of milk used for children nutrition few hundred kilometers away from Chernobyl contain levels of Cs-137 significantly exceeding set limit values. Feeding cows with rotten grass from wet terrain which contains elevated concentrations of radioactive material, increases contamination of milk that directly endangers children. The main problem is the control of foodstuffs. Those sold in shops are controlled by state laboratories, but food sold “on the street” nobody controls. Lack of financial resources for the organization and implementation of large-scale monitoring is an essential problem of establishing control<sup>1</sup>.

A special type of population exposure comes from forest wood used for heating. The ash created by burning wood is used for spreading on arable land, and contains twenty times more Sr-90 than timber sample. This further contaminates agricultural land, and indirectly, all agricultural products that are grown on these plots. In conditions of incurred contamination when no one controls the forests they represent a big risk because of the possibility of the occurrence of forest fires. In the period after the accident over 1,000 forest fires were formed. The resulting ash and smoke contain radionuclides which increase air pollution<sup>1</sup>.

In case of Fukushima Daiichi accident a lucky circumstance was that most of the radioactive material, as much as 80%, was deposited in the Pacific Ocean. In Fukushima accident 140,000 residents have been evacuated: 78,000 were instantly evacuated from the immediate area surrounding the NPP, and later 62,000 from zones that were subsequently contaminated by radioactive cloud<sup>8</sup>. Contamination control of water, milk and food was immediately organized. In the first days after the accident leading contaminant in food and water was I-131, so the iodine prophylaxis was recommended. Although there was sufficient provision, distribution and the beginning of prophylaxis were late and have started on the fourth day, when majority of inhabitants of the contaminated territory have already been evacuated<sup>16,17</sup>. As in the case of the Chernobyl accident, these zones are uninhabited and thus should stay for a long time<sup>18</sup>.

A few days after the accident the direction of the air current changed so that about 20% of the released radioactivity contaminated terrestrial territories of Japan northwest of the NPP, which is much smaller contaminated area than in the Chernobyl accident. These areas are mountain forested landscapes that are not densely populated, houses are scattered and often surrounded by forest. The inhabitants of these regions, 62,000 of them were evacuated during the accident and the majority is still in temporary accommodation with regular social benefits from the government of Japan<sup>19</sup>. Evacuation of the population was not well organized, reported even as chaotic. Residents were relocated several times from place to place, they often rejected transfer, and transportation of immobile patients and old people was not well organized. Many old and immobile remained on contaminated ground. All this has led to a loss of confidence in the authorities of Japan and new actions<sup>20</sup>. In the same way as in the vicinity of Chernobyl residents can be further contaminated. Unlike Chernobyl accident when Soviet government

delayed ban on the consumption of food, the Japanese government immediately banned the use of all foods from this area and provided financial compensation<sup>6,21</sup>.

The level of contamination is still high and the inhabitants of these villages, including children, could receive annual doses of 20 mSv/y, which is the limit for occupationally exposed persons by international standards. It is significantly higher than the dose for the members of the public in non-accidental situations (1 mSv/y), and the dose in the Belarusian villages (5 mSv/y). In spite of that, Japan government made decision to apply the limit for occupationally exposed persons on occupants of this area starting from 2018, when it will abolish all social benefits to displaced persons. The decision was made because the government cannot finance decontamination of this area which is roughly estimated to more than 50 billion USD. Therefore, around 55,000 residents would be forced to return to their contaminated homes. This risk is considered to be unacceptable, especially for children<sup>22</sup>.

From both cases it can be concluded that the contaminated territories remain unusable for people because of their direct impact on health and impossibility to produce basic crops. Evacuation of the population due to contamination represents economic and social collapse of the region. Decontamination, monitoring and regular controls are extremely expensive and, as they should be organized tens, even hundreds of years, not economically feasible. These costs cannot be beared by countries of less economic power (Ukraine and Belarus), nor could be by economically the most powerful country in the world (Japan). Attitudes of state institutions on these issues are often contradictory to attitudes of independent professional organizations.

### *Health effects*

There is a lot about health effects that we still do not know. However, it is certain that there is no unified position on the extent and type of health effects, primarily due to our incomplete knowledge of low-level dose effects. It is almost certain that we will never know all about them, because of lack of the dosimetry results and accurate epidemiological data which are necessary to make a judgment.

#### Acute health effects

During the Chernobyl accident the most exposed was personnel of the NPP and rescuers (firefighters, army and medical personnel). Over 1,000 people have been engaged at the NPP but they neither possessed equipment for dosimetry, nor for personal dosimetry in accidental conditions. Fire and medical teams found themselves on the spot after 15 min. As the dose in the control room were several hundred Gy/h, and at the facility over 100 Gy/h, people involved were exposed to huge doses, and due to acute radiation syndrome first patients were hospitalized during the first hour. Local doses were up to 20 times higher. According to official reports during the first 12 hours 132 people were hospitalized. With the suspicion of acute radiation illness 273 people were hospitalized, while the diagnosis was confirmed in 132, and 28

workers had lethal ending<sup>23</sup>. Extraordinary efforts and the introduction of innovations in the diagnosis and therapy of acute radiation illness ("heroic therapy") half lethal dose (LD-50) increased from 3.25 Gy to 10 Gy. In June 1986 specialized hospitals in Moscow, Minsk and Kiev were established for the treatment of the Chernobyl accident victims<sup>5</sup>. It must be pointed out that there are indications and some authors claim that the number of injuries in the acute phase was several times higher than indicated in the official reports<sup>24</sup>.

Among the residents of the nearest town of Pripjat there were no cases of acute radiation illness. However, the doses were high, even 1,000 times higher than natural. At the night of the accident 40,000 residents were evacuated in only 3 h from the circle of 10 km and a few days after 150,000 residents from the circle of 30 km. Prophylactic distribution of potassium iodide was organized for the residents of Pripjat within 12 h, but it was not organized for the residents of the wider circle of the contaminated zone, which is considered one of the greatest failures of the health system during the accident<sup>15,24,25</sup>.

Residents support by health services consisted of 2,000 physicians, 4,000 technicians, and 1,200 final year medical students. They did more than 600,000 examinations, 215,300 on children, a huge number of laboratory analyzes (radiometric, biochemical, hematological, immunological and cytogenetic)<sup>15,26</sup>.

Fukushima Daiichi accident was induced by natural disasters, earthquake and tsunami that brought widespread destruction of material goods (losses exceeding \$ 200 billion) and a large number of human casualties (15,900 dead and 2,600 missing). A large number of deaths and injuries had nothing to do with radioactive contamination that followed<sup>1</sup>.

As already mentioned, the evacuation and care were not satisfactory, which can be explained by devastation caused by natural disasters. In ordinary situations, Japan is a country with an extremely organized radiation medicine, so that person injured in a radiation accident can be transported to a specialized institution within two hours.

#### Long-term health effects

Long-term effects of Chernobyl accident are the main point of controversy for 30 years. They should be expected in workers exposed to high doses, as well as inhabitants, no matter if evacuated or left at in their homes in the contaminated area. The fact is that after Chernobyl accident more than 600 million people live in less or more contaminated environment<sup>24</sup>. Different ways and time of exposure, dose, dose rate, type of radiation are some of factors influencing onset of late-effects. Radiation-induced late-effects such as malignant diseases, cytogenic changes, and congenital anomalies were in the focus. By time, non-radiation effects came in focus because of their high incidence and their influence on quality of life. At last, social and psychological changes should not be neglected. People evacuated from contaminated zones are higher suicidal, depressive or anxious, abuse alcohol and drugs, have very low birth-rate, etc<sup>1,24</sup>.

Based on Hiroshima victims data, WHO and IAEA estimated that Chernobyl accident could induce additional can-

cers in exposed population in the next years depending on the latent period – from 5 years (for leukemia) to 30 years (for solid tumors). Registers of Ukraine and Belarus, as well as many authors do not agree with the estimation and point out that the risk is highly underestimated. According to these national Registers general cancer morbidity in most exposed regions of Belarus and Ukraine increased 40% and 22% till 2000, respectively. In both countries a higher increase was in children above 17 years and liquidators<sup>27, 28</sup>. According to these data, additional radiation-induced cancers just in Belarus could be up to 62,500 in the next 70 years<sup>29</sup>.

Thyroid cancer is one of the main malignant diseases in Chernobyl victims. It is predominantly, almost always papillary type, very aggressive, fast growing and early metastatic tumor. The highest rate was noticed in children of Belarus 10 years after with 43-fold rate from 1989 to 1994, and 200-fold than before the accident<sup>30</sup>. The increase in thyroid cancer rate has been noticed in contaminated European countries, but in a much lower rate. It is estimated that total number of thyroid cancers could be from 47,000–140,000 cases<sup>29</sup>. Similar situation is noticed with leukemia. After shorter latent period (from few months to 7 years) the incidence increased in all population groups, with the highest rate in children of mostly contaminated regions. The same trend was observed in contaminated European countries (1.5-fold till 1987 in Germany, 2.5-fold in Greece, 37% in Great Britain)<sup>25, 31</sup>.

A high radiosensitivity in children is correlated with high incidence of congenital malformations, lower-birth weight, thyroid gland diseases, leukemia, genetic changes. The number of healthy children is still decreasing every year<sup>26, 31</sup>. The same kind of disturbances, but with lower rate was noticed in many other European countries, such as Finland, Great Britain, Hungary, Turkey, Sweden)<sup>32, 33</sup>.

The increased incidence in morbidity (accelerating age, cardiovascular and respiratory diseases, immunological disorders, diseases of urogenital system, and disturbances of central nervous and musculoskeletal system, cataract, and diseases of the thyroid gland, especially cancer, leukemia) is noticed in adults as well as in children. The incidence rate of these diseases increased 3–4% *per year*, and is higher than before the accident, or in people from uncontaminated zones. Ten years after, 94% of liquidators are not healthy. All the effects mentioned above are dose-dependent, and noticed in other species as well, and therefore cannot be attributable to posttraumatic stress disorder (PTSD) or some other psychological disturbance<sup>24</sup>.

It is the endless list of diseases and statistics, proving one or another attitude. The fact is that all the risk assessments are connected with some kind of financial compensation, state or political decisions, and above all, global attitude towards nuclear energy. Combined with the lack of data (dosimetric or epidemiological) it is ideal soil for ongoing debates and pro- and contra- controversies. The risk should not be neglected, and follow-up studies and long-term monitoring are necessary.

### Serbia

What is the situation in our country? A number of questions are imposed: What are the consequences of the

Chernobyl accident in our country today? How much have we learned from the Chernobyl accident? How much have we done to improve protection of our people?

The Chernobyl accident significantly affected the environmental conditions in our country. The territory of the former Yugoslavia was on the path of the radioactive cloud. Unfavorable circumstances were heavy rain showers that have contributed to a significant deposit of radionuclides. Immediately after the accident systematic measurements of environmental samples in our country began. Measurements were performed by laboratories of many relevant institutions. Most laboratories performed gamma-spectrometry measurements by scintillation (NaI) or semiconductor high-purity germanium (HPGe) counters of high sensitivity<sup>34</sup>.

Measurements were carried out on samples of soil, air, water, milk and dairy products, many foodstuffs, as a primary source of contamination for the population at different locations throughout the country. In addition, measurements included the so-called bioindicators of radiation contamination, such as moss, lichen, grass, honey, mushrooms and others. Based on the measurement results, the transfer of radionuclides was estimated in certain areas of soil, to grass, then to milk, and, at the end to man. In the vast majority of the samples elevated levels of I-131 and Cs-137 were detected. Thus, for example, the exposure rate in Belgrade during the accident was elevated 5 times, until the end of the year and remained 2 times higher; in the region of Tara, Zlatibor and Durmitor it was 2–3 times higher<sup>35</sup>. Natural waters (Danube) have also been contaminated, as well as sediments, maintained after the accident for 20 years<sup>36</sup>.

During the accident the population was kept informed about the kind of food contaminated. Acute health effects were not noticed except a widespread worry and anxiety about health, especially children. A few years later the increase in the number of hematological malignant diseases, and later on thyroid gland, breast, lung, digestive tract malignancies were noticed. Diseases are diagnosed in younger age and in more aggressive forms than before the Chernobyl accident.

Due to direct impact of I-131 and positive epidemiological data from other regions, special attention is attracted by the increased incidence of thyroid cancer. According to data from Cancer Registry for Serbia the incidence of thyroid carcinoma in Serbia is constantly growing, at a rate faster than for any other malignancy. The most common type is papillary thyroid carcinoma in all age groups and both sexes (more in women than in men 3.3 : 1). Data for Serbia do not differ significantly from those of other European countries<sup>31, 37</sup>. Serbia would be considered as a country with medium rate for women and low rate for men. Particularly disturbing is the fact that the largest increase is among women aged 20–29 years. Iodine deficiency as a possible cause can be excluded, because Serbia is not a region deficient in iodine and has standardized iodination of table salt<sup>38</sup>. The increase incidence of malignant diseases, congenital anomalies, immunological and metabolic disorders was noticed in children.

Serbia has no NPPs, but can be threatened by an accident in a nuclear facility abroad. At 1,000 km from the borders of Serbia there are 21 NPPs with 44 reactors, of which 6

NPPs with 12 reactors at 500 km from the border. The closest NPPs are located in Hungary, Romania, Slovenia and Slovakia. All belong to the older generation NPPs. In case of accidents in these NPPs, and appropriate weather conditions, the territory of Serbia may be contaminated in a few hours. The specific weather conditions can lead to high contamination of the territory of Serbia very quickly.

The NPP PAKS 2 is planned to replace two of the four existing nuclear reactors. Hungarian project is very important for all neighboring countries, especially Serbia, due to its proximity to the Serbian border of about 100 km and the direct connection to Danube. Therefore, it is reasonable to discuss the cross-border environmental pollution and the impact on health of the population. Respecting international regulations the Government of Hungary has sent the Risk Assessment Act to 30 countries for discussion. This document discusses the risk zone of 500 m around the NPP and cross-border risks in the ordinary work of NPP. The act alleged to NPP PAKS 2 will not adversely affect environmental conditions in Serbia: it will not pollute the air (none of the polluters), will not affect the water quality of the Danube (no composition, no temperature), the terrestrial and aquatic wildlife, and the environment of the community. The half of the involved countries had complains and asked for international revision of the Act <sup>39</sup>.

*Is Serbia ready to react in the case of nuclear accident and potential emergency situations?*

Serbia is a signatory to the International Convention on Early Warning of Radiation Accidents and the Convention on Mutual Assistance in case of accident. For early notification on the territory of Serbia a system of 9 fixed stations is organized to measure intensity of ambient equivalent dose of gamma radiation. All data are collected and processed by the Serbian Radiation Protection and Nuclear Safety Agency (SRPNA) to other countries. The National Plan for Response in Case of Radiation Emergency is under jurisdiction of the Agency, too.

The National Plan for Response in Case of Radiation Emergency has been in draft version for several years.

A change is needed due to reorganization of the state administration sector, which is transferred from the Ministry of Defence to Ministry of Interior. This service cannot meet all the requirements of an accidental situation due to the lack of material and personal resources and need immediate activity of other relevant services. A whole series of problems showed up, and IAEA has offered help of expert mission that reviewed the plan, facilities, equipment and organization of the whole system planned in the said draft plan. IAEA experts analyzed the draft and pointed out the flaws of the plan with the representatives of relevant institutions.

The crucial part of the National Plan is emergency care and treatment of the injured. This part of the plan is under the jurisdiction of the Ministry of Health. According to the experience of previous nuclear accidents, it would be necessary that the Ministry of Health: make a detailed plan of engagement of medical institutions and a strict division of debts in order to avoid chaotic activity; facilitate the work of emergency teams which, are not equipped, nor trained, for a nuclear accident response; it should organize and provide training and necessary equipment; enable continuous medical assistance to evacuees; establish the conditions and modalities of iodine prophylaxis, from the procurement, storage and distribution of potassium iodide according to defined priorities; develop the guidance on timescales for the application of iodine to current recommendations (a few hours before exposure to 6 h after); to publish detailed instructions for the preparation of doses for children; organize triage and decontamination; ensure continuing education and training for nuclear accident response to all medical staff; take care of food and water- based on the results of measurements to ban or authorize use of water and food.

The very same questions were burning 10 years ago, when we marked 20 years of the Chernobyl accident <sup>40</sup>.

Some progress in preparedness for nuclear emergency was accomplished, but unacceptably slowly. A lot of unsolved problems are still to be solved. We hope that lessons learned from previous accidents in NPPs, and construction of new nuclear reactors in front of our doors, will be sufficient impulse for urgent action.

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## Henoch-Schönlein purpura associated with *Strongyloides stercoralis* infection

Henoch-Schönlein purpura udružena sa infekcijom *Strongyloides stercoralis*

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### Abstract

**Introduction.** Henoch-Schönlein purpura (HSP) is a small blood vessel vasculitis, which usually manifests during childhood. The exact cause of the disease is unknown. **Case report.** We reported a 14-year-old girl who had been admitted to our clinic due to the appearance of red macules on her extremities and face, vomiting, and pain in the abdomen and joints. The patient was initially diagnosed with Henoch-Schönlein purpura. At the end of the fourth week of illness, larvae of *Strongyloides stercoralis* were detected in stool samples. The patient was therefore treated with mebendazole, after which all symptoms permanently withdrew. About a month later laboratory examinations were repeated demonstrating increasing signs of renal damage. Kidney biopsy was performed, showing mesangioproliferative glomerulonephritis with crescents and IgA and C3 positive staining in the mesangium. Upon reviewing the clinical presentation, biochemically demonstrated progressive renal damage and biopsy results, the patient was diagnosed with HSP nephritis. **Conclusion.** The time course of the disease and present knowledge concerning the pathogenic mechanisms of HSP suggest that *Strongyloides stercoralis* infection could have caused HSP in the presented patient, which was complicated by nephritis.

**Key words:** purpura, shoenlein-henoch; nephritis; strongyloidiasis; diagnosis, differential.

### Apstrakt

**Uvod.** Henoch-Schönlein purpura (HSP) je vaskulitis malih krvnih sudova i obično se manifestuje u detinjstvu. Tačan uzrok bolesti nije poznat. **Prikaz bolesnika.** Prikazali smo 14-godišnju devojčicu koja je hospitalizovana na našoj klinici zbog pojave crvenih makula na ekstremitetima i licu, povraćanja i bola u trbuhu i zglobovima. Kod bolesnice je inicijalno postavljena dijagnoza Henoch-Schönlein purpura. Krajem četvrte nedelje bolesti, detektovane su larve nematode *Strongyloides stercoralis* u uzorcima stolice. Bolesnica je stoga lečena mebendazolom, nakon čega su se trajno povukli svi simptomi. Oko mesec dana kasnije ponovljeno je laboratorijsko ispitivanje koje je pokazalo izražene znake bubrežnog oštećenja. Učinjena je biopsija bubrega i uočen mezangioproliferativni glomerulonefritis sa polumesecima uz pozitivno bojenje na IgA i C3 u mezangijumu. Nakon analize kliničke slike, biohemijski potvrđenog progresivnog oštećenja bubrega i rezultata biopsije, kod bolesnice je postavljena dijagnoza HSP nefritisa. **Zaključak.** Vremenski sled događaja i trenutna saznanja o patogenetskim mehanizmima u HSP, ukazuju na to da je infekcija strongiloidesom mogla izazvati HSP koja se komplikovala razvojem nefritisa.

**Ključne reči:** purpura, šenlajn-henohova; nefritis; strongiloidijaza; dijagnoza, diferencijalna.

### Introduction

Henoch-Schönlein purpura (HSP) is a small blood vessel vasculitis, which usually manifests during childhood and is characterized by the presence of immunoglobulin A1 (IgA1) deposits<sup>1,2</sup>. HSP is a self-limiting, systemic, non-granulomatous vasculitis with multiorgan manifestations. The exact cause of the disease is unknown. HSP is the most common vasculitis in childhood<sup>3–5</sup>. Renal affection is the most important aspect of the disease, which determines the

outcome and is most responsible for HSP morbidity and mortality rates. Approximately 40% of HSP patients will develop nephritis during the first 4 to 6 weeks of illness<sup>2</sup>. HSP in children progresses to end-stage renal failure in 1% of patients<sup>6–8</sup>.

### Case report

A 14-year-old girl was admitted to our clinic, due to the appearance of red macules on her extremities and face, vom-

ting, and pain in the abdomen and joints. Skin changes soon became partially confluent, transforming into dark purple papules, which did not blanch on compression and did not cause itching. The patient was previously healthy, and from *anamnesis vitae* we learned about allergic reactions to pollen, nutritive allergens and medications. Additionally to the purpuric rash, the physical exam revealed diffuse abdominal tenderness and tenderness of her large joints in the lower limbs. Laboratory tests performed on admission, showed elevated leukocyte count ( $14.0 \times 10^9/L$ ) with a polymorphonuclear predominance in the formula (94.1%), and increased erythrocyte sedimentation rate (25 mm/h). Basic biochemistry, urine test, ultrasound and radiography examination findings were normal. Due to intense abdominal pain, surgical treatment was considered on several occasions, but physical exam and additional testing did not justify surgical intervention. During the first week of illness, we found elevated blood pressure (above 95 percentile for sex, age and height), elevated level of total serum IgE (182.90 kIU/L), positive proteins (1+) in the morning urine sample along with microhematuria. In the second week of illness, fresh blood was noted in her stool, which did not appear in the later course of disease. Upon admission the patient was treated with corticosteroids, initially with methylprednisolone, then prednisone, with antihistamine and hypoallergenic diet. Introduction of corticosteroids mitigated abdominal and joint pain. During the third week of illness skin changes completely withdrew and the patient had no other symptoms. In the beginning of the fourth week of the illness, the patient had another burst of purpuric rash on her face, forearms and feet, and complained of abdominal pain, while still on corticosteroid treatment. Repeated tests showed reduced levels of complement components in serum (C3 0.02 g/L, C4 0.0 g/L) and significant proteinuria, (1.6 g/24h) with normal creatinine clearance in the 24-hour urine collection test. Total blood cholesterol, proteins and other biochemical test values were within the reference range. Serum levels of  $\alpha$ ,  $\gamma$  and  $\mu$  class of immunoglobulins were also normal each time they were measured. At the end of the fourth week of illness, rhabditiform and filariform larvae of *Strongyloides stercoralis* were detected by direct microscopic examination of stool samples. Mebendazole therapy was initiated accordingly to these findings. After mebendazole treatment abdominal symptoms withdraw completely and there were no new bursts of purpuric rash. About a month later the patient received another course of mebendazole therapy and laboratory examinations were repeated demonstrating increased serum cholesterol level (7.42 mmol/L), positive proteins in the morning urine sample (2+), increased proteinuria (2.92 g/24h) with normal creatinine clearance in the 24-hour urine collection test, and normal serum levels of C3 and C4. Based on these results a kidney biopsy was performed, showing mesangioproliferative glomerulonephritis with crescents and immunohistochemical staining positive for IgA and C3 deposits in the mesangium. Upon reviewing the clinical presentation, biochemically demonstrated progressive renal damage and biopsy results, the patient was diagnosed with Henoch-Schönlein purpura nephritis. The patient was then treated with combined therapy, consisting of prednisone, azathiopri-

ne and enalapril. After a month of treatment a complete urinary remission of the disease was achieved, while her cholesterol levels were still elevated. Later evaluation demonstrated normal biochemistry results, apart from persistently increased serum IgE level (256.25 kIU/L).

### Discussion

The presented patient was initially diagnosed with Henoch-Schönlein purpura according to the 2010 EULAR/PRINTO/PRES<sup>1</sup> (EULAR – The European League Against Rheumatism; PRINTO – Paediatric Rheumatology International Trials Organisation; PRES – Paediatric Rheumatology European Society) criteria<sup>9</sup>, based on characteristic palpable purpuric rash without thrombocytopenia, associated with pain in the abdomen and joints and signs of kidney damage. The disease manifested itself dominantly by pronounced gastrointestinal signs, along with biochemically demonstrated progressive renal damage. The treatment with systemic corticosteroids significantly mitigated gastrointestinal symptoms, but could not prevent the development of nephritis as demonstrated in previously published reports<sup>10</sup>. It is well known that corticosteroids can alleviate the symptoms but do not affect the course of the disease, thus prednisone use is not recommended for prevention of persistent renal disease<sup>11,12</sup>. Henoch-Schönlein purpura aetiology is unclear, but possible causes might be bacterial, viral and parasitic infections<sup>13-17</sup>, alterations in secretion of interleukins (interleukin 1 and 6)<sup>18</sup> or growth factors (platelet derived growth factor, transforming growth factor  $\beta$ )<sup>19</sup>, as well as vaccination (vaccines for cholera, measles, yellow fever, *Salmonella typhi* and *paratyphi* A and B)<sup>20</sup>. There are evidence to support the correlation of disease severity with increased serum levels of thrombin-antithrombin complexes, prothrombin factors 1 and 2, von Willebrand antigen and D-dimer<sup>21</sup>. Certain alleles are attributed to increasing likelihood of HSP<sup>22</sup>. The presented patient had several episodes of rash associated with abdominal pain and a brief period of intermittent appearance of fresh blood in the stool. After detection of *S. stercoralis* larvae in stool samples and upon administering mebendazole, there were no further bursts of rash nor gastrointestinal complaints. Based on the present knowledge, *S. stercoralis* may be considered as the initiator of HSP in the presented patient.

The key role in the pathogenesis of HSP most probably belongs to abnormal IgA1<sup>23</sup>. The main origin of aberrantly glycosylated IgA1 are the mucosal tissues and bone marrow<sup>24-27</sup>. Therefore, we suggest that infection caused by *S. stercoralis* provoked the production of IgA1 in the intestinal mucosa, which preceded the forming and precipitation of immune complexes containing aberrantly glycosylated IgA1 leading to the development of vasculitis and clinical manifestations of HSP. However, our patient's urine examination showed microhematuria and increasing proteinuria in the 24-hour urine collection test. The presence of galactose deficient gA1 (Gd-IgA1) makes the best distinction between HSP patients who will develop nephritis and those who will not. HSP patients with nephritis have been shown to have increased serum levels of IgG specific for Gd-IgA1, as oppose to

HSP patients without nephritis and healthy controls<sup>28,29</sup>. The present view is that antiglycan antibodies recognize Gd-IgA1 and form immune complexes that precipitate in the mesangium and then give rise to the process of renal damage by activating complement cascade and initiating leukocytoclastic vasculitis, a process indistinguishable from that in IgA nephropathy<sup>30</sup>.

Decreased expression of  $\beta$ 1,3-galactosyltransferase and increased expression of  $\alpha$ 2,6-sialyltransferase has been detected in HSP patients with nephritis, unlike HSP patients without nephritis<sup>31,32</sup>. IgA1 molecules of healthy people have mono- or disialylated galactosamine-N-acetylgalactosamine (Gal-GalNAc) disaccharide, thus it is possible IgA1 with non-sialylated galactose or N-galactosamine participate in the pathogenesis of HSP nephritis<sup>33,34</sup> since the clearance of immune complexes containing IgA1 depends on asialoglycoprotein receptor expressed by Kupffer cells<sup>35</sup>. Propensity towards the development of nephritis is linked to HLA-B35 allele<sup>36</sup>. According to previously stated research results, there is a certain genetic predisposition of some HSP patients to develop nephritis.

Recent papers describe cases with *S. stercoralis* infection leading to renal damage and the development of nephrotic syndrome<sup>37,38</sup>, but direct infection of renal parenchyma has not been demonstrated in neither of those cases. Pathohistological findings of our patient's kidney biopsy matches those seen in HSP nephritis<sup>39</sup>, thus damage by direct infection is unlikely. Later reevaluation of the presented patient's medical status reveals that serum IgE level was still increased, which could be attributed to her atopic constitution. Total IgE is frequently elevated in HSP patients<sup>40</sup>, and association of HSP with type I hypersensitivity reactions has been so far documented in several studies<sup>41,42</sup>. During the course of her illness, the patient never had loose stools, maldigestion, eosinophilia, nor any other symptom of infection with an intestinal parasite except abdominal pain, so the abdominal pain might be considered as the only symptom of *S. stercoralis* infection. Abdominal pain is one of the most common manifestations of HSP and about two-thirds of patients complain of this symptom<sup>43</sup>, so if we attribute this symptom to HSP, the infection itself could be considered asymptomatic. The presented patient complained of abdominal pain in spite of early introduction of corticosteroids, and the complete withdrawal of symptoms was achieved only after treatment with mebendazole, indicating the significance of strongyloidiasis for the development of HSP in the presented case. Corticosteroids provide for quick resolution of symptoms in HSP patients<sup>44-46</sup>, however, patients with strongyloides infection receiving im-

munosuppressive drugs, and especially corticosteroids, may develop severe hyperinfection or disseminated infection syndrome<sup>47-49</sup>. *S. stercoralis* is a nematode leading a complex life cycle. It is the only helminth to secrete larvae in faeces and continuously reproduces within the host, therefore *S. stercoralis* is able to maintain long-lasting persistence by the process of autoinfection causing mild symptoms (cough, bloating, loss of appetite) or no symptoms at all in the majority of infected people<sup>50</sup>. Corticosteroids contribution to the propagation of the autoinfection process could not be ruled out in the presented patient. It also needs to be emphasized that the use of corticosteroids did not lead to clinical presentation of severe hyperinfection or disseminated infection syndrome, but it is probable that corticosteroid treatment created the conditions for *S. stercoralis* reproduction and increasing their number, which made possible microbiological detection of larvae in stool samples.

*S. stercoralis* can persist for more than 30 years without developing a clinically notable disease<sup>51-53</sup>. The risk for severe strongyloidiasis exists for all patients treated with corticosteroids that come in contact with this nematode, while the duration of corticosteroid use is highly variable (4 days to 20 years), as shown by Fardet et al.<sup>47</sup> and is therefore not a major determinant. Having in mind the life cycle and the lack of symptoms in most infected people, we could not know when the presented patient became infected. Laboratory examinations of the presented patient demonstrated biochemical signs of renal damage during the first week of illness, and since strongyloidiasis clinical course could be with no symptoms, it is possible that pre-existing strongyloides infection could have caused nephritis, although there are no known mechanisms for parasitic infection to influence the early onset of nephritis in HSP. Some published reports demonstrate remission of nephrotic syndrome after strongyloides eradication<sup>37,54,55</sup>, implying a causal link between strongyloidiasis and glomerulonephritis.

## Conclusion

Strongyloidiasis is not a severe disease in immunocompetent people and its association with HSP has not been previously described. Resolution of clinical signs of HSP in our patient was achieved after treatment with mebendazole, but this intervention did not stop the development of nephritis. The time course of the disease and present knowledge concerning the pathogenic mechanisms of HSP, suggest that *Strongyloides stercoralis* infection could have caused HSP in the presented patient, which was further complicated by nephritis.

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## Double orifice mitral valve – A case report

### Dvostruki orificijum mitralne valvule

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#### Abstract

**Introduction.** Double orifice mitral valve (DOMV) is a very rare congenital heart defect. **Case report.** We reported 20-year-old male referred to our center due to evaluation of his cardiologic status. He was operated on shortly after birth for a tracheoesophageal fistula. Accidentally, echocardiography examination at the age of 4 years revealed double orifice mitral valve (DOMV) without the presence of mitral regurgitation, as well as mitral stenosis, with normal dimensions of all cardiac chambers. The patient was asymptomatic, even more he was a kick boxer. His physical finding was normal. Electrocardiography showed regular sinus rhythm, incomplete right bundle branch block. Transthoracic echocardiography (TTE) examination revealed the normal size of the left atrial, mitral leaflets were slightly more redundant. The left and right heart chambers, aorta, tricuspid valve and pulmonary artery valve were normal. During TTE examination on a short axis view two asymmetric mitral orifices were seen as a double mitral orifice through which we registered normal flow, without regurgitation and mitral stenosis. Transesophageal echocardiography (TEE) examination from the transgastric view at the level of mitral valve, showed 2 single asymmetric mitral orifices separated by fibrous tissue, mitral leaflet with a separate insertion of chordae for each orifice. **Conclusion.** The presented patient with DOMV is the only one recognized in our country. The case is interesting because during 16-year a follow-up period there were no functional changes despite the fact that he performed very demanded sport activities. This is very important because there is no information in the literature about that.

#### Key words:

heart defects, congenital; diagnosis; echocardiography, transesophageal.

#### Apstrakt

**Uvod.** Dvostruki orificijum mitralne valvule (DOMV) je vrlo retka kongenitalna srčana mana. **Prikaz slučaja.** Prikazali smo mladića starog 20 godina, aktivnog sportistu, kik boksera, koji je upućen u Centar za kardiologiju Kliničkog centra Crne Gore, u cilju redovne provere kardiološkog statusa. Neposredno nakon rođenja bolesnik je operisan zbog traheozofagealne fistule. Rutinski ehokardiografski pregled u četvrtoj godini života pokazao je do tada neprepoznati DOMV, bez prisustva stenozе ili regurgitacije i bez drugih anomalija na srcu. Na pregledu kod kardiologa za odrasle, u 20. godini života mladić je bio bez ikakvih tegoba. Objektivnim pregledom nalaz na srcu bio je uredan. Elektrokardiografom registrovan je sinusni ritam, nekompletni blok desne grane. Na poprečnom transtorakskom ehokardiografskom pregledu viđena su dva asimetrično postavljena otvora bez prisutne regurgitacije ili stenozе. Ostali nalaz bio je uredan. Transezofagusnim ehokardiografskim (TEE) pregledom u nivou mitralne valvule ustanovljena su 2 asimetrična mitralna orificijuma odvojena fibroznom tkivom, mitralni kuspisi uz separatnu inserciju hordi za svaki orificijum. **Zaključak.** Prikazani bolesnik je jedini dijagnostikovani bolesnik u Crnoj Gori. Posebno je značajna činjenica da za vreme praćenja od 16 godina i aktivnog bavljenja vrlo zahtevnim sportom nisu nastale funkcionalne promene na srcu. Takođe, ne postoje preporuke u literaturi za ovaj problem.

#### Ključne reči:

srce, kongenitalne mane, dijagnoza; ehokardiografija, transezofagusna.

#### Introduction

Double orifice mitral valve (DOMV) is a very rare congenital heart defect. Greenfield<sup>1</sup> was the first author who described this heart defect in 1876. Until today about 200 cases of do-

uble orifice mitral valve were described<sup>2</sup>. Approximately 50% cases of double orifice mitral valve are detected during investigation of other congenital heart disease<sup>2</sup>.

Mitral valve consists of a central orifice located between the sail-like anterior leaflet and a C-shaped posterior

mitral leaflet. The anterior cusp occupies roughly one third of the annular circumference and the posterior leaflet occupies about the remaining two-thirds of the annular circumference<sup>3</sup>. DOMV is a defect with two separate mitral orifices separated by fibrous tissue<sup>2</sup>. Pathoanatomic substrate are two openings. Usually in 85% of cases the eccentric orifice located on anterolateral or posteromedial commissure. In the minority of cases there is a central type and fibrous tissue that separates the two symmetrical holes<sup>3</sup>. In DOMV the mitral valve is functioning normally in about 50% cases and in the other 50% cases the valve is functioning like stenotic or there is regurgitation<sup>4</sup>. Some authors describe a duplicate of the mitral valve: this condition involves two mitral valves and each of them with its own set of leaflets, commissures, chordae and papillary muscles<sup>5</sup>. Double orifice mitral valve was found in 1% of autopsied cases of congenital heart disease in the Cardiac Registry of the Children's Hospital, Boston<sup>6</sup>. Postmortem, in 27 cases an anomaly of the tensor apparatus was always found<sup>6</sup>. These malformations may be associated with the chordal ring, accessory papillary muscle or muscles, subdividing muscular ridge, fused papillary muscles (parachute mitral valve), crossing chordae tendines, and central fibrous subdivision<sup>6</sup>. The embryologic theories explaining this abnormal leaflet fusion and persistence of the left part of the common atrioventricular canal. Abnormal structure, including large bridging tissue, abnormal leaflets, chordae fused or abnormal papillary muscles, reduce the effective area and valves can result in clinically significant degrees of mitral incompetence<sup>7,8</sup>. DOMV without mitral regurgitation or mitral stenosis is asymptomatic<sup>9</sup>. Surgical intervention is necessary when stenosis or incompetence is severe or if repair of an associated cardiac lesion is needed<sup>9</sup>.

A total of 46 children, aged 2 days to 16 years with DOMV were studied and partial atrioventricular septal defect was the most commonly associated cardiac lesion<sup>10</sup>. Symptoms were related to the degree of mitral insufficiency and/or stenosis when present. Surgical intervention directed at double orifice mitral valve was required in the minority of patients who underwent repair of associated cardiac lesions. The long-term morbidity attributable to DOMV was low<sup>10</sup>. Other associated lesions include atrial septal defects (ASD), ventricle septal defect (VSD), coarctation of the aorta, tetralogy of Fallot, patent ductus arteriosus, hypoplastic left heart syndrome, left superior vena cava, unroofed coronary sinus, Ebstein anomaly of the tricuspid valve, dysplastic tricuspid valve, double-orifice tricuspid valve, Shone syndrome, parachute mitral valve, flail mitral-valve leaflet, truncus arteriosus, pulmonary stenosis, bicuspid aortic valve, left ventricular noncompaction (isolated and nonisolated types), tricuspid atresia, and corrected transposition<sup>10-19</sup>.

### Case report

The presented 19-year-old patient male is the only one patient registered in our country, which has 650,000 inhabitants. The congenital heart disease, DOMV, was accidentally discovered 4 years after the operation due to tracheoesopha-

geal fistula. It was the isolated form without the presence of mitral stenosis and mitral regurgitation, with the normal dimensions of all the cardiac chambers. At the age of 19 years, he was examined by the adult cardiologist. He had no complaints. He was an active athlete – kick boxer. Physical finding was normal, ECG showed regular sinus rhythm with incomplete right bundle branch block. Chest X ray demonstrated the normal size of the heart. Transthoracic 2D and 3D echocardiography was performed with a Phillips HD11XE ultrasound machine.

Transthoracic echocardiography (TTE) examination registered in sectional parasternal long axis view: normal left atrium size, mitral leaflets slightly more voluminous, the left ventricle of completely normal size without segment changes into kinetic with preserved systolic function. The root of the aorta was of normal width, aortic valve with three cusps, leaflet thin with good separation. The ascending aorta was of completely normal width (Figure 1). On sectional parasternal short axis view there was a double mitral orifice, mitral valve was divided into two separate valve orifices (anterolateral and posteromedial) by a fibrous bridge through which registered normal flow (Figure 2).



Fig. 1 – Long axis view – without abnormalities.

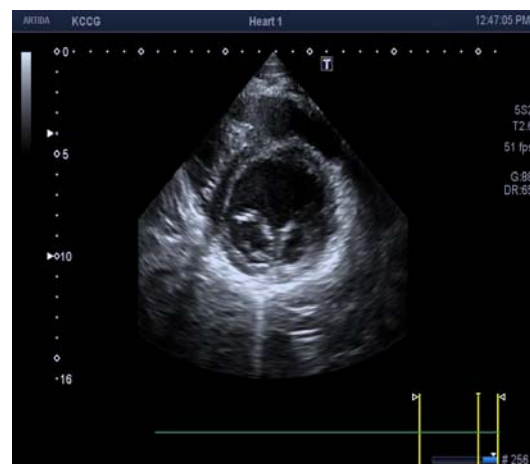


Fig. 2 – Short axis view – double mitral valve orifice.

On the apical 4-chamber view, the central part of the anterior mitral leaflet, the fibrous bridge did not move toward the apex at diastole while the medial and lateral parts did (Figure 3). Four-chamber view of the tricuspid valve and pulmonary artery valve looked normal. A trace of tricuspid regurgitation



and systolic pressure were registered in the right ventricle (RVSP) of 20 mmHg. Right cavities were completely normal. The pericardium was normal (Figure 3). Transesophageal echocardiography showed the transgastric view two separate asymmetric mitral orifice separated by fibrous tissue, mitral leaflet with a separate one for each insertion hordes orifice (Figure 4). Blood flow through both the orifices was normal. No other congenital cardiac anomaly was detected.



**Fig. 3 – Four chamber view – unusual view of the mitral valve during systole; center part of the anterior mitral leaflet, the fibrous bridge did not move toward the apex at diastole while the medial and lateral parts did.**



**Fig. 4 – Transesophageal echocardiography (TEE) transgastric view – unequal holes in the double orifice mitral valve.**

## Discussion

DOMV is a rare congenital heart disease. Based on echocardiography recording Trowitzsch et al.<sup>5</sup> described 3 types of DOMV: hole type (accessory orifice surrounded by leaflet tissue that may have a chordal ring), complete bridging (fibrous bridge in the plane of the mitral valve sails, dividing the mitral valve opening into two parts that may be equal or unequal), and incomplete bridging – a small strand of fibrous tissue connects only the tips of the anterior leaflets separator insertion hordes of every orifice. The presented patient had unequal, asymmetric position of orifices.

The mitral valve is functionally normal in about 50% of patients and significant regurgitation or stenosis is found in the remaining patients<sup>8</sup>. In a published clinical series of 46 children with DOMV, the authors reported mitral regurgitation as the most frequent association, detected by color Doppler in 43% of the patients<sup>10</sup>. DOMV with normal flow was seen in 37% of the patients, and mitral stenosis in 13% of the patients<sup>10</sup>. Physical findings may only exist if mitral regurgitation or mitral stenosis are present<sup>20</sup>. The presented patient had an isolated form without the presence of mitral stenosis and mitral regurgitation, so physical finding was normal.

Associated congenital heart defects are common, although DOMV can occur as an isolated anomaly. The most common associated lesion is atrioventricular septal defect found in 4.9–17.9% of cases in necropsy studies<sup>5</sup>. The presented patient had no associated anomalies.

Double orifice mitral valve has also been reported in patients presenting with atrial tachycardia or congenital complete heart block<sup>18, 19</sup>. The presented patient had no rhythm disturbance. The problem is that there is no recommendation for patients with DOMV could they be actively engaged in sport. Physical activity restriction is recommended for symptomatic patients<sup>21</sup>.

Treatment depends on the type and severity of mitral valve dysfunction. Isolated DOMV causing neither obstruction nor regurgitation needs no active intervention<sup>9</sup>. Long-term follow-up is necessary to detect abnormal hemodynamics or subsequent complications<sup>22</sup>. Mitral stenosis and regurgitation were found together in 6.5% of the patients, but only 16% of the patients had severe enough to require surgical lesions intervention<sup>10</sup>. According to the updated guidelines of the American Heart Association, patients with double orifice mitral valve do not need antibiotic prophylaxis against infective endocarditis<sup>21</sup>. Patients with DOMV with mitral stenosis are amenable to percutaneous transcatheter balloon dilatation<sup>23</sup>. Surgical repair should be performed in all symptomatic patients with DOMV which has regurgitation and should also be performed in patients with stenotic DOMV, if balloon dilation is not feasible<sup>21</sup>. Follow-up care is necessary for prompt detection and treatment of infections, arrhythmia and other complications<sup>21</sup>.

## Conclusion

The presented patient with DOMV is the only one recognized in our country. It is the isolate form of this rare disease, asymptomatic, without associated anomalies, as well as without mitral regurgitation and mitral stenosis. The case is interesting because during a 16-year follow-up period there were no functional changes despite the fact that he performed a very demanding sport activity. This is highly important because there is no information in the literature about that.

## Declaration of interest

All the authors declare no conflict of interest.

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## Secondary venous aneurysm following intravenous drug abuse: A case report

### Sekundarna venska aneurizma kao posledica intravenozne zloupotrebe narkotika

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#### Abstract

**Introduction.** Venous aneurysm (VA) is a rare condition that can be presented in both superficial and deep venous system. Secondary VAs as well as pseudoaneurysms are usually caused by external spontaneous or iatrogenic trauma. They are often misdiagnosed and inadequately treated. Complications include thrombosis, phlebitis, eventual pulmonary embolism and rupture. **Case report.** We presented a case of secondary VA of the great saphenous vein developed in a young addict following chronic intravenous drug application in the groin region. Aneurysm required urgent surgical treatment due to bleeding complication as it was previously misdiagnosed for hematoma (or abscess) and punctuated by a general surgeon. Complete resection of VA with successful preservation of continuity of the great saphenous vein was performed. Postoperative course was uneventful. Regular venous flow through the great saphenous vein was confirmed on control ultrasound examination. **Conclusion.** VAs are uncommon, among them secondary VA being extremely rare. In cases with a significant diameter or threatening complications surgical treatment is recommended.

#### Key words:

aneurysm; saphenous vein; diagnosis, differential; histological techniques; injections, intravenous; opioid-related disorders; vascular surgical procedures

#### Apstrakt

**Uvod.** Venska aneurizma je retko patološko stanje koje se može pojaviti kako na površnom, tako i na sistemu dubokih vena. Sekundarne venske aneurizme, kao i venske pseudoaneurizme, najčešće se javljaju kao posledica spoljne, neretko jatrogene traume. S obzirom na nisku učestalost, one se često ne prepoznaju i shodno tome nepravilno leče. Moguće komplikacije uključuju trombozu, flebitis, plućni tromboembolizam, pa čak i rupturu. **Prikaz bolesnika.** Prikazali smo sekundarnu vensku aneurizmu lokalizovanu na velikoj veni safeni kod mlade narkomanke, kao posledicu dugotrajnog uzimanja intravenoznih narkotika preko prepone. Aneurizma je zahtevala hitno hirurško lečenje usled akutne hemoragijske komplikacije, zbog toga što je prethodno bila punktirana pod dijagnozom suspektog hematoma, odnosno apscesa, od strane opšteg hirurga. Učinjena je potpuna resekcija aneurizme, uz očuvanje kontinuiteta safenske vene. Postoperativni tok je protekao bez komplikacija, a uredna protočnost vene safene potvrđena je kontrolnim ultrasonografskim pregledom. **Zaključak.** Venske aneurizme su retke, naročito one sekundarne etiologije. Ukoliko dosegnu značajan prečnik ili prete komplikacijama, potrebno je hirurško lečenje.

#### Ključne reči:

aneurizma; v.saphena; dijagnoza, diferencijalna; histološke tehnike; injekcije, intravenske; poremećaji izazvani opioidima; hirurgija, vaskularna, procedure.

#### Introduction

Venous aneurysm (VA) is a rare pathologic condition. The first was described in the popliteal vein by May and Nisnel<sup>1</sup> in 1968. It can be presented in both superficial and deep venous system. Primary superficial VA is considered to be a

consequence of venous reflux distal from a valve<sup>2</sup>. In contrast, primary deep VA is thought to appear due to venous wall weakness, as in Klippel-Trenaunay syndrome<sup>3</sup>. Both superficial and deep secondary VAs (and pseudoaneurysms) are caused by external spontaneous or iatrogenic trauma<sup>4-6</sup>. Due to their low incidence VAs are often misdiagnosed and

inadequately treated. In such cases complications can occur and include thrombosis, thrombophlebitis with eventual pulmonary embolism and rupture.

### Case report

A 27-years-old female was admitted as emergency due to hemorrhage from a tumefaction in the left groin. The patient had a history of chronic intravenous drug abuse repeatedly using both groins for drug-consuming access. This tumefaction appeared three months earlier and was punctuated as suspected hematoma (or abscess) by a general surgeon in a regional hospital before admission. Because of subsequent massive bleeding a superficial skin suture was applied and followed by compressive bandaging of the groin. The patient was thereafter referred to a vascular specialist.

On admission compressive bandage was removed and a soft, bluish, painful, non-pulsating tumefaction without thrill or bruit about  $4 \times 4$  cm in diameter was observed. All the arterial pulses in the leg were palpable. As the mass was localized medially from femoral artery pulsation a venous pseudoaneurysm of saphenous or femoral vein was suspected. Due to ultrasound unavailability during emergency hours

the indication for surgery was established on the basis of clinical examination.

Under the regional anesthesia a midgroin incision was made and the region of saphenofemoral junction was accessed. A VA of a terminal part of great saphenous vein was completely exposed as well as unaffected parts of saphenous and femoral vein (Figure 1).

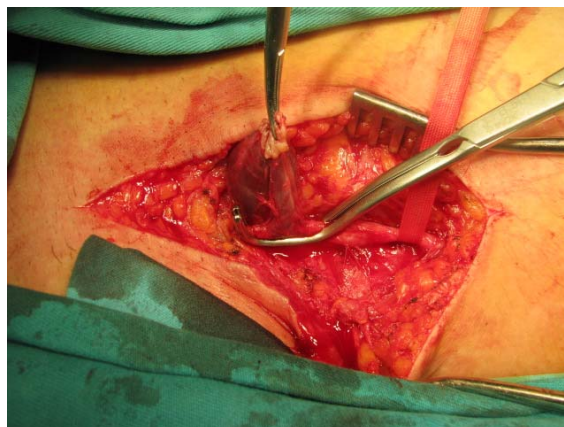
Except the small punctiform lesion on the top of the aneurysm no other defects were noted. There were no signs of suppurative collections or extensive hematomas. Systemic heparin was administered and partial clamp of the saphenofemoral junction applied, followed by complete aneurysm resection (Figure 2). The junction defect was closed with direct lateral suture, in order to preserve superficial venous flow.

The patient recovered well and was discharged on the second hospital day. Control ultrasound presented patent saphenofemoral junction on discharge. No pathological reflux or signs of previous thrombosis were found. However, the patient has never appeared to control examination.

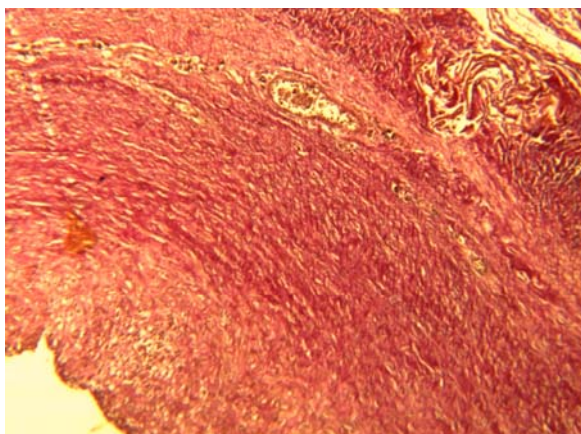
Histological examination revealed VA with signs of wall arterIALIZATION, irregularly intimal and medial fibrosis and thickening (Figure 3). Scattered disruption and lack of elastic and smooth muscle fibers and diffuse accumulation of



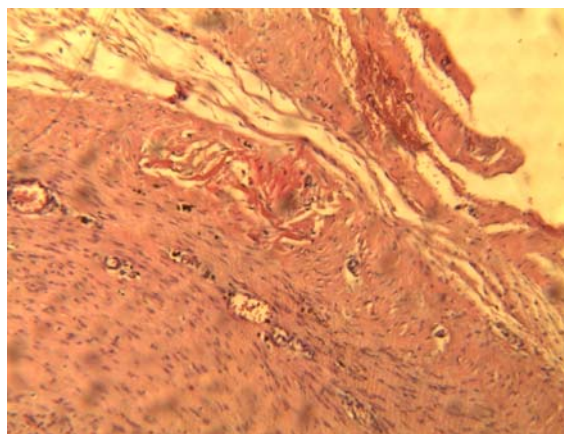
**Fig. 1 – Intraoperative finding of venous aneurysm engaging the terminal part of the great saphenous vein.**



**Fig. 2 – Partial clamping of the saphenofemoral junction enabling safe aneurysm resection.**



**Fig. 3 – Histological finding of resected venous aneurysm shows signs of wall degeneration, irregularly intimal and medial fibron's and thickening (Van Giemsa,  $\times 40$ ).**



**Fig. 4 – Histological finding of resected venous aneurysm shows scattered disruption and lock of elastic and smooth muscle fibers and diffuse accumulation of extracellular matrix (Van Giemsa,  $\times 400$ ).**

extracellular matrix was also noted (Figure 4). Nevertheless, all three layers of the venous wall were persisting in the sac, thus suggesting the presence of a true aneurysm.

### Discussion

VAs are mostly reported in sporadic cases, many of them pronounced as “the first”, “unique” etc. Indeed, there are only a few series including a significant number of patients being published<sup>2,7</sup>. However, the definition of VA as a measurement exceeding 1.5 times the adjacent proximal or distal vein in one of these series was quite liberal<sup>2</sup>. Unfortunately there are no universally agreed size criteria to define a venous dilatation as an aneurysm<sup>8</sup>.

Histological findings of VA usually describe a significant decrease in the number and size of smooth muscle cell, decreased elastic fibers and thickening and fibrosis of the intima layer<sup>9</sup>. The terms “endophlebohypertrophy” and “endophleboscclerosis” are used to describe these changes<sup>10</sup>. Although posttraumatic in nature, histological evaluation of our patient favored a true VA because all layers of venous wall were present.

There is also a topographic distribution of VAs on central (thoracic), visceral, cervical and peripheral<sup>11</sup>. Differential diagnosis of superficial VA includes pseudoaneurysms, arteriovenous malformations, enlarged lymph nodes, lipomas and varicose veins<sup>9</sup>. In most cases the correct diagnosis is established after additional investigation, duplex ultrasound being usually sufficient. Our case confirms the diagnostic challenge of VAs as it has been unrecognized previously and punctuated as inguinal hemathoma. It outlines the value of duplex ultrasound in preoperative evaluation however in emergency it is not always available. For that reason medical practitioners should be aware of this rare but potentially serious disease.

The management and indications for operative treatment of this disorder remain unclear. It is obvious that large VAs threatening with complications should be treated surgically; however, there is no consensus about the treatment of small and asymptomatic VA. Some authors recommend observation, compression and surveillance with duplex

scanning, or percutaneous less invasive treatment<sup>2,12</sup>. Furthermore, there is no strict definition between “small” and “large” VA that would help in establishing indication for surgery. Sessa et al.<sup>7</sup> estimated the diameter of popliteal VA greater than 20 mm to be clinically significant and should be treated by surgery.

There is a variety of conventional surgical approaches to VA depending on their localization, size, and eventual presence of complications. In VA engaging superficial veins the most simplex procedure is complete removal and ligation, however in our case we preserved a saphenofemoral junction by VA excision and lateral suture due to patient’s young age, in order to keep this main superficial venous outflow tract patent. If VA engages deep venous system, every attempt to restore venous flow should be made including the use of synthetic graft<sup>13</sup>. The role of postoperative anticoagulation is not yet established and is left to surgeon’s preference. In our case we did not administer any postoperative anticoagulation, due to social profile of our patient. However, she never appeared on control exam.

VAs and pseudoaneurysms in the groin following drug addiction are not commonly reported, only one small series of patients being published<sup>14</sup>. All of them were treated with complete excision and wound package due to the significant presence of infection. Only one case of iatrogenic true aneurysm of the superficial antecubital vein has been reported and was related to venous cannulation<sup>8</sup>.

### Conclusion

Venous aneurysms are uncommon, among them secondary venous aneurysms being extremely rare. In cases with significant diameter or threatening complications surgical treatment is recommended.

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## Autologous hematopoietic stem cell transplantation in combination with immunoablative protocol in secondary progressive multiple sclerosis – A 10-year follow-up of the first transplanted patient

Autologna transplantacija matičnih ćelija u kombinaciji sa imunoablativnim protokolom kod sekundarne progresivne multiple skleroze – 10 godina praćenja prvog transplantiranog bolesnika

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### Abstract

**Introduction.** Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system that affects young individuals and leads to severe disability. High dose immunoablation followed by autologous hematopoietic stem cell transplantation (AH SCT) has been considered in the last 15 years as potentially effective therapeutic approach for aggressive MS. The most recent long-time follow-up results suggest that AH SCT is not only effective for highly aggressive MS, but for relapsing-remitting MS as well, providing long-term remission, or maybe even cure. We presented a 10-year follow-up of the first MS patient being treated by immunoablation therapy and AH SCT. **Case report.** A 27-year-old male experienced the first symptoms - intermittent numbness and paresthesia of arms and legs of what was treated for two years by psychiatrist as anxiety disorder. After he developed severe paraparesis he was admitted to the Neurology Clinic and diagnosed with MS. Our patient developed aggressive MS with frequent relapses, rapid disability progression and transition to secondary progressive form 6 years after MS onset

[the Expanded Disability Status Scale (EDSS) 7.0 Ambulation Index (AI) 7]. AH SCT was performed, cyclophosphamide was used for hematopoietic stem cell mobilization and the BEAM protocol was used as conditioning regimen. No major adverse events followed the AH SCT. Neurological impairment improved, EDSS 6.5, AI 6 and during a 10-year follow-up remained unchanged. Brain MRI follow-up showed the absence of gadolinium enhancing lesions and a mild progression of brain atrophy. **Conclusion.** The patient with rapidly evolving, aggressive, noninflammatory MS initially improved and remained stable, without disability progression for 10 years, after AH SCT. This kind of treatment should be considered in aggressive MS, or in disease modifying treatment nonresponsive MS patients, since appropriately timed AH SCT treatment may not only prevent disability progression but reduce the achieved level of disability, as well.

**Key words:** multiple sclerosis, chronic progressive; hematopoietic stem cell transplantation; transplantation, autologous; treatment outcome; magnetic resonance imaging.

### Apstrakt

**Uvod.** Multipla skleroza (MS) je imunski posredovana bolest centralnog nervnog sistema koja ugrožava mladu populaciju i dovodi do teških invaliditeta. Imunoablativna terapija u kombinaciji sa autolognom hematopoetskom transplantacijom matične ćelije (ATMĆ) poslednjih 15 godina smatra se potencijalno efikasnim terapijskim pristupom za agresivnu formu MS. Najnoviji rezultati dugotrajnog praćenja pokazuju da ATMĆ nije efikasna samo za visokoagresivnu MS, već i za relapsno-remitentni oblik MS, obezbeđujući dugotrajnu remisiju, možda

čak i izlječenje. Prikazali smo rezultate 10-godišnjeg praćenja prvog bolesnika sa MS koji je lečen imunoablativnom terapijom i ATMĆ. **Prikaz bolesnika.** Bolesnik, star 27 godina, imao je prve simptome – povremenu ukočenost i parestezije ruku i nogu, zbog kojih je tokom dve godine lečen kod psihijarata pod dijagnozom anksiozni poremećaj. Zbog teške parapareze primljen je na Kliniku za neurologiju, gde je dijagnostikovana MS. Prikazani bolesnik oboleo je od agresivnog oblika MS sa čestim relapsima. Invaliditet je brzo napredovao i nakon šest godina od početka bolesti nastupio je prelazak u sekundarno progresivni oblik: proširena skala funkcionalne onesposoblje-

nosti (EDSS) 7.0, Ambulation Index (AI) 7. Urađena je ATMĆ. Korišćen je ciklofosamid za mobilizaciju matičnih ćelija iz krvi, a BEAM protokol kao kondicioni režim. Nije bilo značajnijih neželjenih dejstava tokom i nakon ATMĆ. Došlo je do poboljšanja neurološkog deficita. Skor EDSS bio je 6.5, AI 6 i tokom 10 godina praćenja ostao je nepromenjen. **Zaključak.** Kod prikazanog bolesnika sa agresivnom, neinflamatornom MS, nakon ATMĆ došlo je do poboljšanja neurološkog deficita i odsustva dalje progresije narednih 10 godina.

## Introduction

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system that affects young individuals and leads to severe disability. Available disease modifying treatment (DMT) such as interferon beta 1a, 1b and glatiramer acetate have proven to be effective in relapse reduction by around 30% in relapsing-remitting (RR) form of MS (RRMS)<sup>1</sup>. However, relapse reduction is not achieved in at least 40–60% of RRMS patients and DMT does not diminish disability progression<sup>1</sup>. Therefore, there is a subgroup of MS patients which deteriorate in spite of DMT, as well as those with aggressive MS which is characterized with a high relapse rate, rapid disability progression and a high magnetic resonance imaging (MRI) activity.

High dose immunoablation followed by autologous hemopoietic stem cell transplantation (AH SCT) has been considered in the last 15 years as potentially effective therapeutic approach for severe cases of MS, RRMS and secondary progressive (SP) MS (SPMS) or those unresponsive to conventional therapies<sup>2</sup>. The most recent long-time follow-up results suggest that AH SCT is not only effective for highly aggressive MS, but for RRMS as well, providing long-term remission, or maybe even cure<sup>3,4</sup>. On behalf of European Bone Marrow Transplantation (EBMT) and European Charcot Foundation, Guidelines for autologous blood and marrow stem cell transplantation in multiple sclerosis were recommended in 2000<sup>5</sup>. The concept of immunoablative therapy and AH SCT is the induction of immune tolerance by resetting immune response in patients with MS, i.e. replacement of the dysfunctional immune system with the newly formed one<sup>4,6</sup>.

We presented a 10-year follow-up of the first MS patient being treated by immunoablation therapy and AH SCT in Serbia.

## Case report

The presented patient, a military officer, experienced first symptoms – intermittent numbness and paresthesia of arms and legs, at the age of 27. He was treated by psychiatrist for 2 years as anxiety disorder. It was after developing severe paraparesis that he was admitted in our clinic and diagnosed with MS, according to the McDonald criteria<sup>7</sup>. In the next 3 years he had 6 relapses, affecting repeatedly brainstem and cerebellar functions. At that time he was under care of other neurologist and DMT was not recommended. He was seen again in our clinic, aged 34, when

Ovu vrstu lečenja treba razmotriti kod agresivne MS, jer na vreme urađena ATMĆ može ne samo da spreči napredovanje invaliditeta, već i da redukuje neurološki deficit.

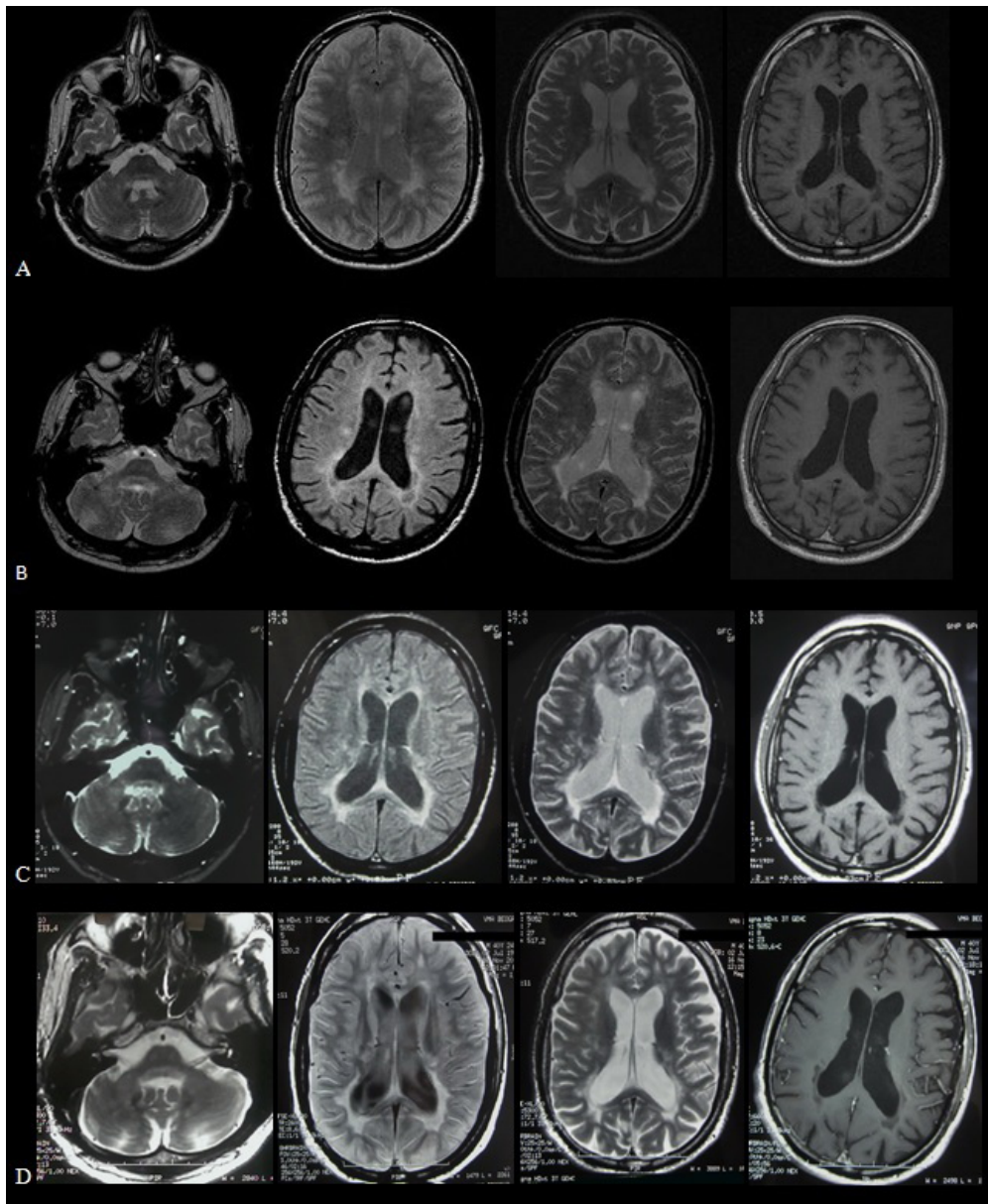
## Ključne reči:

**multipla skleroza, hronična, progresivna; transplantacija hematopoeznih matičnih ćelija; transplantacija, autologna; lečenje, ishod; magnetna rezonanca, snimanje.**

the course of MS was secondary progressive and the Expanded Disability Status Scale (EDSS) 6.0<sup>8</sup>. At that point, DMT would not be justified and since he deteriorated rapidly – EDSS worsening by 1 point during 6 months, mitoxantrone (MTX) treatment was started. He received 12 mg/m<sup>2</sup> MTX, every 3 months, 3 times. During this treatment he further deteriorated for 0.5 point EDSS, reaching 6.5. At that point, AH SCT was considered and thorough conversation was performed with the patient and his parents. He was informed about the procedure, potential risks and expected results. Ethical Committee of the Military Medical Academy, Belgrade, approved procedure and the patient signed informed consent in May 2005. Neurological examination revealed mild-moderate head tremor at rest, internuclear ophthalmoplegia, severe intention tremor of the arms, leg ataxia, as well as trunkal ataxia with inability to sit unless supported. Global muscle strength was preserved, deep tendon reflexes were symmetric and slightly hyperactive and plantar response was normal. His speech was slurred and he had difficulties while swallowing (he was able to drink on a straw and to eat only blended food). Gait was severely ataxic and he was able to walk with support of another person around 10 m. Vibration sense was lost at the level of the right rib frame and markedly decreased at the same level on the left side. Bowel, bladder and sexual functions were intact. Cognitive testing could not be performed considering limb ataxia and slurred speech. Overall, EDSS was 7.0, ambulation index (AI) 7<sup>9</sup>, quality of life scale, Functional Assessment of Multiple Sclerosis being used<sup>10</sup>, showed low scores on all the six domains tested – mobility, symptoms, emotional well-being, general content, thinking and fatigue, family and social well being. He was otherwise healthy. Physical exam was normal, as well as chest X-ray and abdominal ultrasound. Dental problems were sanated. ECG showed sinus rhythm, f72/min. All laboratory analysis needed for transplantation procedure were within normal range; magnetic resonance imaging (MRI) of the brain showed multifocal demyelinating lesions extensively within the supra- and infratentorial white matter, including juxtacortical and periventricular regions. The majority of the lesions were in the chronic stage with a diffuse gliotic reaction. No gadolinium enhancing lesions were detectable. High grade atrophy, predominantly affecting the white matter was noted, with no significant regional predominance or asymmetry (Figure 1 A).

Peripheral blood stem cells were mobilized by using cyclophosphamide (4 g/m<sup>2</sup>) and human granulocyte colony-stimulating factor 10 mg/kg body weight. Intensive





**Fig. 1 – a) Baseline magnetic resonance imaging (MRI) findings: axial T2 (TR 3241, TE 100), axial PD (TR 3241, TE 33), axial T2 (TR 3241, TE 100), axial T1 post-contrast (TR 610, TE 12). High-grade brain atrophy, infratentorial and periventricular white matter demyelinating lesions, no post-contrast enhancement are shown; b) MRI at the 2-year follow-up: axial T2 (TR 2100, TE 100), axial PD (TR 2100, TE 20), axial T2 (TR 2100, TE 100), axial T1 post-contrast (TR 613, TE 12). Mild progression of brain atrophy, a few new demyelinating lesions, no post-contrast enhancement; c) MRI at the 3-years follow-up: axial T2 (TR 2200, TE 95), axial PD (TR 2200, TE 35), axial T2 (TR 2200, TE 95), axial T1 post contrast (TR 500, TE 14). Slight progression of brain atrophy, no new demyelinating lesions, no contrast enhancement; d) MRI at the 10-year follow-up: axial T2 (TR 5300, TE 72), axial PD (TR 2600, TE 86), axial T2 (TR 5300, TE 72), axial T1 post-contrast (TR 660, TE 20). Mild brain atrophy progression mainly infratentorial, no new demyelinating lesions, no post-contrast enhancement.**

rehydration, alkalization and mesna (Uromitexan<sup>®</sup>) protection were used during cyclophosphamide infusion. A total nucleated cell and mononuclear cell (MNC) count was determined by flow cytometry (Technicon H-3) and 260 mL of cell suspension with  $12.9 \times 10^8$  MNC/kg was collected. For autologous stem cell cryopreservation, our own controlled-rate freezing protocol was carried out<sup>11</sup>. No adverse events occurred during mobilization. The patient was discharged from the hospital in good health, with normal complete blood

count (CBC) and biochemistry analysis, without signs of infection. The BEAM protocol was used as conditioning regimen – BCNU (carmustine) 30 mg/m<sup>2</sup>, ARA-C (cytarabine) 800 mg/m<sup>2</sup>, etoposide 800 mg/m<sup>2</sup>, melphalan 140 mg/m<sup>2</sup>, according to well-known and established oncological procedure. At the same time, in order to prevent side effects, the patient received antiviral, antibacterial and antifungal treatment. On day 0, AHSCT was performed and the patient received 260 mL cell suspension with  $12.9 \times 10^8$  MNC/kg

intravenously without side effects and without hemolysis. During posttransplantation period, on days +1 and +2, the patient received rabbit antithymocyt globulin 7.5 mg/kg. The most pronounced myelosuppression was noted on day +7, white blood cells (WBC)  $0.03 \times 10^9/L$  (normal range  $4.00\text{--}11.00 \times 10^9/L$ ), platelets was  $10 \times 10^3$  (normal range  $150\text{--}400 \times 10^9/L$ ), followed by petechial changes on skin and oral mucosa. The patient received six units of concentrated platelets and two units of concentrated erythrocyts. Since day +4 he was febrile (up to  $38.2^\circ\text{C}$ ) and after isolation of coagulase-negative *Staphylococcus* form, the patient received antibiotics according to antibiogram. He was febrile during 6 days, without clinical and neurological deterioration. Repeated hemocultures remained sterile and the patient was discharged from the hospital, two weeks after transplantation, in good health. He had the same EDSS 7.0, CBS showing WBC  $16.4 \times 10^9/L$  with neutrophilia predominance, Hgb 102 g/L (normal range 110–180 g/L), red blood cells (RBC)  $3.71 \times 10^6$  (normal range  $4.4\text{--}5.8 \times 10^{12}/L$ ), platelets  $98 \times 10^9/L$ . Oral aciclovir was used as antiviral prophylaxis for six months post transplantation. Oral trimethoprim-sulfamethoxazole for *Pneumocystis* infection prophylaxis was continued for 6 months post-transplantation in alteration with ciprofloxacin. Antifungal prophylaxis with oral nystatin solution was continued for 6 months after the transplantation. He also continued with clonazepam for intention tremor. In subsequent monthly visits patient's CBC completely recovered, while neurological improvement was noted 6 months post-transplantation. These include ability to swallow hard food, decrease in head tremor at rest, decreased intention tremor of arms, absence of truncal ataxia and comprehensible speech, which resulted in the EDSS decrease of 0.5, i.e. EDSS 6.5, AI 6. He was seen initially every 3 months, then every 6 months during the second and the third year and then yearly. No adverse events related to transplantation were noted. No relapses were recorded, his neurological status remained unchanged, EDSS 6.5, AI 6, marked improvement was noted on the quality of life scale, in subscales of general content, emotional well-being, additional concerns and social well-being. At a 10-year follow-up, EDSS was 6.5, AI 6. Brain MRI follow-up was performed after 2, 3 and 10 years (Figure 1, B–D).

## Discussion

Our main expectations of immunoablation and AHSCT were related to disease stabilization – the absence of new relapses and sustained progression of disability. During a 10-year follow-up period the presented patient did not have relapses, nor disability progression. We even observed improvement in certain neurological functions 6 months after AHSCT which were sustained throughout the whole 10-year post transplantation period. These neurological improvements resulted in EDSS decrease of 0.5, better ambulation and marked improvement on the quality of life scale.

Our findings are in concordance with the recently published results on the long-term follow-up of AHSCT in MS<sup>12–14</sup>. In the reported results progression-free survival (PFS) at 15 years was 44% in patients with active/inflammatory disease and 10% in those without inflammation, such as the presented

patient<sup>13</sup>. After 5 years, EDSS improvement/stabilization was confirmed in 66% patients and after 7 years in 48% patients, including RR and SPMS patients<sup>14</sup>. The presented patient improved and decreased EDSS score for 0.5 six months post AHSCT which sustained for 10 years. Mancardi et al.<sup>14</sup> showed EDSS improvement of 0.5–1.0 within the first year after AHSCT, sustained for the subsequent years in 37% SPMS patients. Sustained EDSS improvement  $> 1$  was confirmed in only 3% of SPMS patients and in 31% of RRMS. All the three published papers showed considerably better AHSCT outcome in RRMS, when inflammation is dominant pathological process and contributes to disability more than neurodegeneration. Neurological impairment caused by inflammation could be reversed by immunosuppression applied in AHSCT protocol, which explains improvement in EDSS score  $> 1$  in one third of transplanted RRMS patients.

A pronounced effect on inflammation is confirmed by MRI results, showing a higher PFS and a better outcome in those with gadolinium+ lesions prior to AHSCT compared to those without MRI activity<sup>13,14</sup>. The progression free survival (PFS) for these patients was found to be between 10%<sup>13</sup> and 46% at 5 years<sup>14</sup>. The presented patient did not have gadolinium+ lesions at baseline and remained progression free during a 10-year follow-up.

Noted improvements in our patient, as well as other SPMS patients reported, may be contributed not only to antiinflammatory effect of this protocol, since there was no MRI visible CNS inflammation, but to the known neurotrophic and neuroprotective effect of hematopoietic stem cell and resolution of conduction block<sup>3,6</sup>.

Transplantation related mortality (TRM) is one of the most important issues regarding AHSCT in MS. During 2001–2007, TRM in MS was reduced to 1.3%, from 7.3% in the previous period 1995–2000<sup>14</sup>. Better patient selection and avoidance of high-intensity conditioning regimens resulted in a marked TRM reduction. There was no TRM in a recently published paper presenting Swedish experience of AHSCT in 52 patients with aggressive MS<sup>3</sup>. No mortality was reported in recent Russian<sup>15</sup> (95 patients) and Czech study<sup>12</sup> (26 patients) as well. Periprocedural toxicity such as fever, viral and bacterial infections or sepsis are not rare, nor insignificant, but they are well-managed in experienced hematological departments licensed for bone marrow transplant. The only periprocedural complications in our patient were related to the period of the maximal myelosuppression, seven days posttransplantation. He had an episode of *Staphylococcus* coagulase negative bacteriemia, successfully treated with antibiotics and marked anemia and thrombocytopenia which needed transfusions. Analysis of all performed AHSCT has shown that older age (over 50 years), higher EDSS score (over 7.0), intense conditioning and/or extensive T-cell depletion protocol increase morbidity and mortality rate<sup>2</sup>.

Concern was raised regarding brain atrophy after AHSCT, which was found to be 6% within the first posttransplant year, but afterwards return to 2% which is usual brain atrophy rate for MS patients<sup>16</sup>. Baseline brain MRI in the presented patient showed marked brain atrophy and a mild atrophy increase was noted 10 years after the transplantation.

## Conclusion

The presented patient with rapidly evolving, aggressive, noninflammatory secondary progressive multiple sclerosis, initially improved and remained stable, without disability progression for 10 years, after the combined immunoablative protocol and autologous hematopoietic stem cell transplantation. This treatment, performed as the palia-

tive procedure led to progression-free survival for 10 years in the presented patient. Autologous hematopoietic stem cell transplantation should be considered in aggressive multiple sclerosis, or in disease modifying treatment nonresponsive multiple sclerosis patients, since appropriately timed autologous hematopoietic stem cell transplantation may not only prevent disability progression but reduce the achieved level of disability, as well.

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**MS Word for Windows** (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office (Excel, Word Graph)**. The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

#### Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References, Enclosures**.

##### 1. Title page

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: \*, †, ‡, §, ||, ¶, \*\*, ††, ...

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

##### 2. Abstract and key words

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background/Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should

contain subtitles **Introduction, Case report, Conclusion**. Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

##### 3. Text

The text of the articles includes: **Introduction, Methods, Results, and Discussion**. Long articles may need subheadings within some sections to clarify their content.

**Introduction.** After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideration of the subject, nor data or conclusions from the work being reported.

**Methods.** The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethics Committee for the tests in humans and animals.

**Results** should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

**Discussion** is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

##### References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al.* Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

##### Examples of references:

*Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinanska-Mladenovska O, Arsov T, Strezova A, et al.* Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. *Vojnosanit Pregl* 2003; 60(6): 657–612.

*DiMaio VJ.* Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

*Blinder MA.* Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

*Christensen S, Oppacher F.* An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

*Aboud S.* Quality improvement initiative in nursing homes: the ANA acts in an advisory role. *Am J Nurs* [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: <http://www.nursingworld.org/AJN/2002/june/Wawatch.htm>

##### Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

##### Illustrations

Any forms of graphic enclosures are considered to be figures and should be submitted as additional databases in the System of Assistant. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1, Figure 2** and so on). If a figure has been published, state the original source.

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#### Primeri referenci:

*Durović BM*. Endothelial trauma in the surgery of cataract. *Vojnosanit Pregl* 2004; 61(5): 491–7. (Serbian)

*Balint B*. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

*Mladenović T, Kandolf L, Mijušković ŽP*. Lasers in dermatology. In: *Karadaglić D*, editor. *Dermatology*. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

*Christensen S, Oppacher F*. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. *Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming*; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

*Abood S*. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. *Am J Nurs [serial on the Internet]*. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: <http://www.nursingworld.org/AJN/2002/june/Wawatch.htm>

### Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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